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Emerging Frontiers of Deep Eutectic Solvents in Drug Discovery and Drug Delivery Systems

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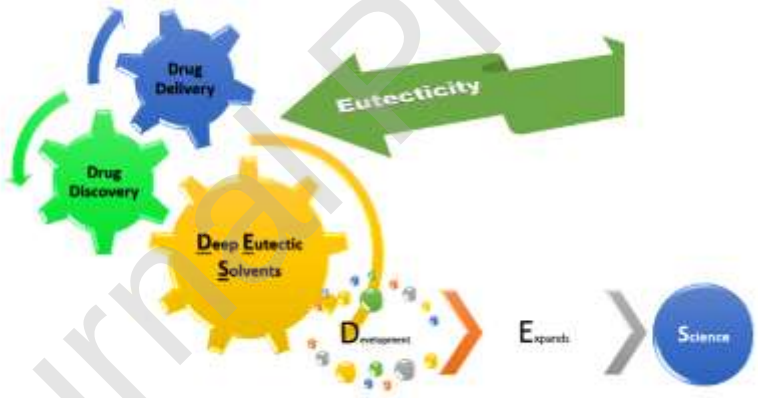
Graphical abstract



Abstract

The applications of eutectic systems, including deep eutectic solvents (DESs), in diverse sectors have drawn significant interest from researchers, academicians, engineers, medical scientists, and pharmacists. Eutecticity increases drug dissolution, improves drug penetration, and acts as a synthesis route for drug carriers. To date, DESs have been extensively explored as potential drug delivery systems on account of their unique properties such as tunability and chemical and thermal stability. This review discusses two major topics: first, the application of eutectic mixtures (before and after the introduction of DES) in the field of drug delivery systems, and second, the most promising examples of DES pharmaceutical activity. It also considers future prospects in the medical and biotechnological fields. In addition to the application of DESs in drug delivery systems, they show greatly promising pharmaceutical activities, including anti-fungal, anti-bacterial, anti-viral, and anti-cancer activities. Eutecticity is a valid strategy for overcoming many obstacles inherently associated with either introducing new drugs or enhancing drug delivery systems.

Abbreviations: Acetaminophen, **ACP;** acetylsalicylic acid, **AA;** acrylic acid, **AC;** active pharmaceutical ingredient, **API**; alanine aminotransferase, **ALT;** aspartate transaminase**, AST;** benzoic acid, **BA;** bovine serum albumin, **BSA**; cannabidiol drug, **CD**; carbon nanomaterial, **CNM;** choline acetate, **ChAc;** choline chloride, **ChCl**; choline bicarbonate and geranic acid, **CAGE**; confocal laser scanning microscopy, **CLSM**; conductor-like screening model for real solvent, **COSMO-RS;** ChCl:maleic acid, **CM**; deep eutectic solvents, **DESs**; deep eutectic solvent derivative, **DESD**; deoxyribonucleic acid, **DNA**; diethylethanolammonium chloride, **DAC;** differential scanning calorimetry, **DSC;** *N,N*-diethylethanolammonium chloride, **EAC;** effective concentration at 50% **EC_{50};** eudragit-based eutectic system, **EES;** fourier-transform infrared spectroscopy, **FTIR;** glucose:sucrose, **GS;** hydrogen bond acceptor, **HBA**; hydrogen bond donor, **HBD**; high performance liquid chromatography, **HPLC**; itraconazole, **ITR**; herpes simplex virus type-1, **HSV-1**; herpes simplex virus type-2, **HSV-2**; human embryonic kidney cells, **HEK-293;** human gastric

cancer cell line, **AGS;** human oral keratinocyte cell, **OKF6;** human malignant melanoma cell line, **A375;** human colon adenocarcinoma cell line**, HT-29;** human prostate cancer cell line**, PC3;** human liver hepatocellular cell line, **HepG2;** human cervical cancer cell line, **HelaS3;** human oral keratinocyte cells, **H413;** lethal concentration at 50%, **LC_{50};** lactate dehydrogenase, **LDH**; lidocaine hydrochloride, **LidHCl;** methacrylic acid, **MAA;** methyltriphenylphosphonium bromide, **MTPPB**; macrophage line, **RAW264.7**; hydroxypropylmethyl, **HPMC**; ionic liquids, **ILs**; human breast cancer cell line, **MCF-7;** median lethal dose, **LD_{50};** minimum inhibitory concentration, **MIC;** ***mycobacterium tuberculosis***, **TB**; n-methylpyrrolidone, **NMP;** natural deep eutectic solvent, **NADES;** nuclear magnetic resonance, **NMR;** polymeric eutectic delivery system, **PES;** phosphate-buffered saline, **PBS;** phenylacetic acid, **PA;** poly(vinyl alcohol), **PV;** potential hydrogen, **pH;** poly(acrylic acid), **PA;** pulsed-field gradient, **PFG;** poly(octanediol-*co*-**citrate) elastomers, POS;** reactive oxygen species, **ROS;** silver chloride, **AgCl;** starch:poly-ε-**caprolactone, SPCL**; triethylene glycol, **TEG;** therapeutic deep eutectic solvent, **THEDES**; ultraviolet-visible, **UV-Vis;** 1-Octyl-3-methy-limidazolium chloride**, [C8mim][Cl]**; 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromidefor, **MTT**.

Keywords: Ionic liquid; anti-cancer; eutectic mixtures; drug dissolution; eutecticity; therapeutic deep eutectic solvent; Pharmaceutics; nanomedicine; nanotechnology; pharmaceutical industry.

1. Introduction

The term “eutectic” is originally from the Greek word for easy or low melting [1]. A eutectic system is defined as a combination of two or more components in a specific ratio that possesses a lower melting point than either alone. Some examples of eutectic solutions are syrup and honey, i.e. highly viscous mixtures of sugars at room temperature. Eutectic systems have been widely used in numerous applications, including the glass and ceramic industries, energy storage devices, and pharmaceutical formulations [2]. In the pharmaceutical field,

eutectic mixtures have long been utilized in numerous applications such as drug delivery, the medium for enzymatic reactions, and drug solubilization [3-5].

Deep eutectic solvents (DESs) are a new generation of eutectic mixtures currently gaining widespread scientific and technological attention as low-cost alternatives for organic solvents and ionic liquids (ILs). These neoteric, green solvents achieve lower melting points than do their single components because of the charge delocalization resulting from hydrogen bonding between the hydrogen bond donor and the halide anion [6-8]. Compared to ILs and other conventional solvents, DESs are recognized as less volatile, thermally stable, highly tunable, biodegradable, less toxic, and lower in cost [4, 9-11]. To that end, DESs have become essential players in various fields of the chemical, biotechnology, and electrochemical industries [12-16].

Recently, a new class of DESs has been synthesized from complexations of primary metabolites or bio-renewable ingredients such as amino acids, sugar alcohols, sugars, and organic acids [17-20]. This novel category, termed "natural deep eutectic solvents (NADES)," has been linked to some previously incomprehensible natural phenomena such as the solubility and biosynthesis of semi-polar components that are neither soluble in water nor a lipid phase [1, 21-23]. NADESs have also been associated with several biological mechanisms for drought resistance, dehydration, germination, and cryoprotection [18, 24].

In particular, the emerging push for green practices among the scientific community has led to a huge demand for the application of NADES in the nutraceutical and pharmaceutical fields. NADESs are touted as great alternatives to hazardous organic solvents in the

separation, extraction, and purification of natural bioactive substances [25]. They have been employed for the extraction of numerous bioactive compounds, such as phenolic acid, polyphenols, flavonoids, polysaccharide, and proteins, from diverse natural sources [26-30]. In addition, certain types of recently-introduced DESs possess medicinal or pharmaceutical activities in their own right, e.g. anti-fungal, anti-bacterial, anti-viral, and anti-cancer activities. The term therapeutic deep eutectic solvent (THEDES) was coined for DESs that contain active pharmaceutical ingredients (APIs) [31, 32].

Another recently-developed type of DES is the deep eutectic solvent derivative (DESD) [29]. This is a DES-like, room temperature liquid that is similar to but distinct from eutectic systems; it is also referred to as a low transition temperature mixture, eutectic mixture, and low melting mixture [33-35]. DESD encompasses an extensive range of DES-like derivatives, especially those derived from ternary systems, for which the eutectic point is difficult to determine. Examples of DESDs reported to date include DESD ChCl:glycolic acid (1:2), DESD ChCl:glycolic acid:oxalic acid (1:1.6:0.4), and DESD ChCl:glycolic acid:oxalic acid (1:1.7:0.3) [29, 36].

Presently, DESs and DESDs are attracting significant interest as drug delivery systems, owing to their high tunability and lower toxicology profiles. It is known that the efficiency of a drug/API is directly correlated to its solubility, permeability, and bioavailability; lower solubility of a drug always leads to lower rates of dissolution and permeation. In addition, an ideal solvent must also prevent the aggregation and precipitation of the drug/API. The properties of DESs and DESDs have great promise for addressing these problems in the development of new drug delivery systems. Hence, this review offers an important

perspective on the in the utilization of eutecticity in drug delivery and drug discovery as reported from 1998 through early 2019. This review will additionally look "one step ahead", mapping current progress and framing the foreseeable future research directions.

2. Drug delivery

2.1. Eutectic mixture

Transdermal delivery of drugs has considerable benefits over conventional therapy, such as improving healing efficiency, ameliorating side effects, and avoiding hepatic first-pass metabolism [3, 37]. However, the use of transdermal delivery systems is restricted by the capability of a drug to penetrate the skin [38]. Drug solubility, molecular weight or size, capability for hydrogen bonding, and lipophilicity are among the factors that play primary roles in determining a drug's transportation efficiency [3]. Conventionally, drugs for transdermal delivery are designed as lipophilic solutions, usually and highly concentrated in order to ensure they permeate the skin membrane efficiently [39]. Typically, the drug is initially dissolved in an oil solution to form an emulsified oil phase. However, this decreases the thermodynamic driving force and, hence, hampers the penetration of the drug into the skin barrier. Some researchers tried to use ethanol as a water-miscible co-solvent [38]. Unfortunately, such a co-solvent always has adverse side effects on the epithelia and skin.

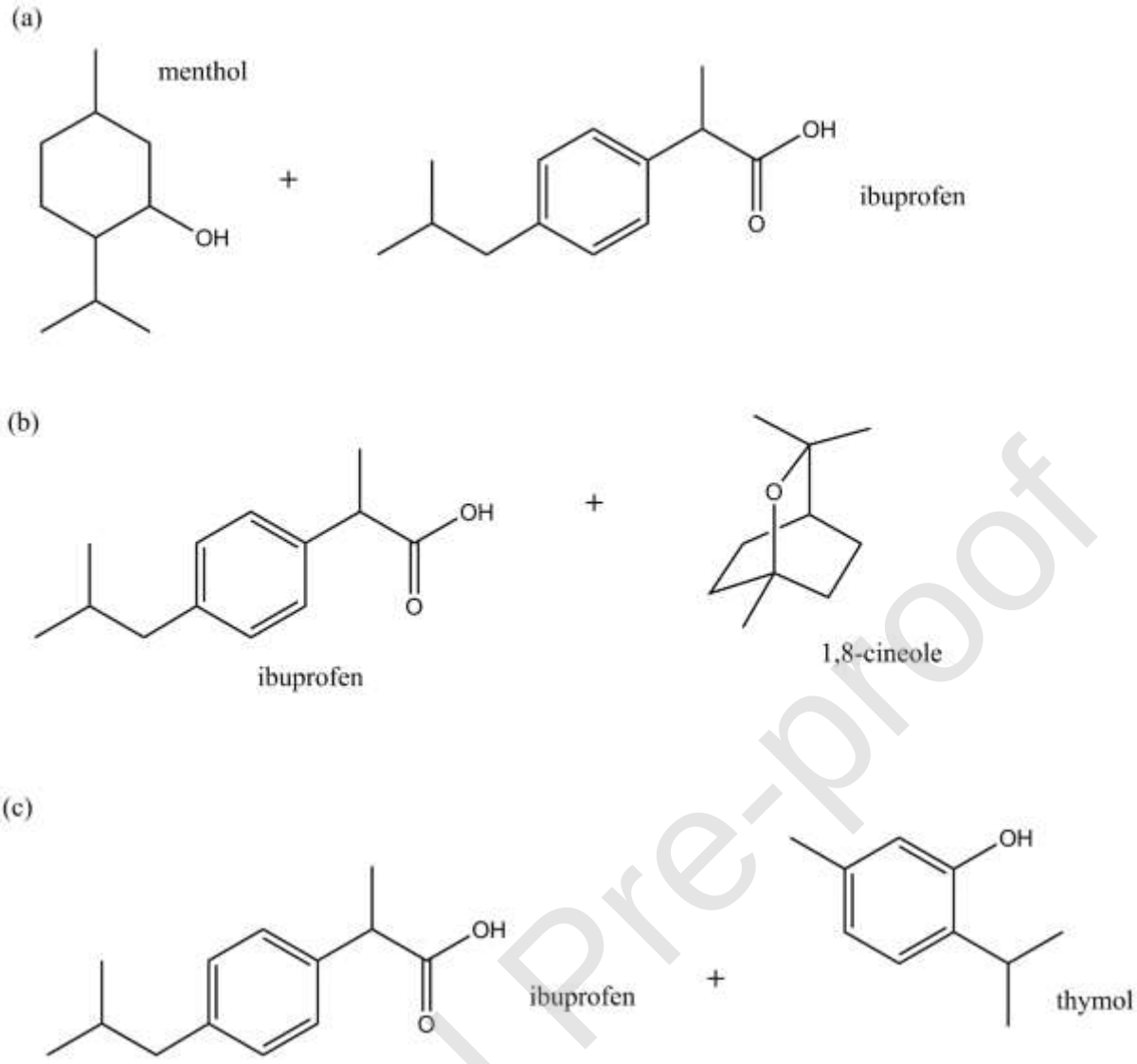
Another approach is to turn the solid drug into a liquid, which is believed to reduce crystallization and the presence of polymorphs, thereby enhancing the drug's bioavailability as well as transdermal delivery [40]. This leads to the utilization of eutectic mixtures in drug permeation applications. To date, significant enhancement of drug efficiency and

bioavailability through improving drug cellular membrane permeation has been demonstrated by a variety of eutectic mixtures [31, 41-43], as will be discussed thoroughly in this section.

2.1.1. Ibuprofen-based eutectic mixtures

Most early studies on drug-based eutectic mixtures revolved around the preparation of ibuprofen [(RS)-2-(4-(2-methylrpopyl)phenyl)propanoic acid]-based eutectic mixtures [3, 39, 43, 44]. Binary eutectic mixtures (Scheme 1) involving ibuprofen and several types of terpenes (e.g. LD-menthol, L-menthol, thymol, and 1,8-cineole) demonstrably increased the transdermal delivery of ibuprofen across Caucasian abdominal skin [3]. The eutectic systems also displayed higher ibuprofen flux compared to treatment with individual components. For instance, ibuprofen:L-menthol (1:1) showed a higher ibuprofen flux, at 31.8 μg cm^{-2} h^{-1}, as than did L-menthol solution alone, at only 13.7 μg cm^{-2} h^{-1} (Table 1). This increased transdermal flux was implicated to result from the melting point depression effect of the eutectic mixture. Comparing two eutectic ratios of ibuprofen: LD-menthol, i.e. 7:13 (w/w) and 1:4 (w/w), showed that increasing the menthol fraction also incremented the ibuprofen flux, from 13.0 μg cm^{-2} h^{-1} to 27.0 μg cm^{-2} h^{-1}. This result suggests that the driving force is increased as the fraction of menthol increases, and hence increasing the menthol content expedites the rate of ibuprofen flux across the membrane. For each binary mixture, maximum ibuprofen flux at 32 ºC was observed when some ibuprofen solid formed (i.e. precipitated) in the solution. Meanwhile, most minimum flux values were observed from homogenous solutions with low ibuprofen formation. This is in agreement with previous reports that the disappearance of solid drug inform a eutectic solution leads to a decrease in the driving force and the thermodynamic activity of drug permeation [39, 43, 44].

In another work, the increase of ibuprofen flux into the membrane was found to be highly correlated with the melting point depression of eutectic mixtures [3]. This finding is supported by the inverse correlation between the melting point of the permeant and transdermal penetration activity observed in a previous study [45]. As melting point decreases, ideal solubility increases, leading to an exponential increase in transdermal flux. Karande and Mitragotri [44] additionally reported that the melting points of drugs and their solubility and lipophilicity in skin are inversely proportionate. This suggests that the permeation of ibuprofen through the skin may increase significantly when it is delivered by a eutectic system.



Scheme 1: Ibuprofen-based DESs e.g., (A) menthol: ibuprofen (3:7) (B) Ibuprofen:1,8-cineole (2:3) (C) Ibuprofen: thymol (2:3)

Table 1: The use of eutectic systems in drug delivery applications.

DES	Ratio	API	Permeation flux (μg cm^{-2} h^{-1})	Permeability coefficient (cm h^{-1})	Diffusivity (cm^2 h^{-1})	Ref.

Ibuprofen:L-menthol	1:1	Ibuprofen	31.8	–	–	[3]
Ibuprofen:LD-menthol	1:1	Ibuprofen	33.8	–	–	[3]
Ibuprofen:LD-menthol	7:13	Ibuprofen	13.0	–	–	[3]
Ibuprofen:LD-menthol	1:4	Ibuprofen	27.0	–	–	[3]
Borneol:menthol	1:3	Daidzein	–	2.67×10^{-7}	–	[46]
ITR:phenol	1:1	ITR	–	22.73×10^{6}	–	[47]
Choline bicarbonate: geranic acid	1:1	Insulin	0.57	–	–	[48]
Choline bicarbonate: geranic acid	1:1	BSA	0.19	–	–	[48]
Menthol:ibuprofen	3:1	Ibuprofen	–	2.3×10^{-6}	–	[49]
Prilocaine: lidocaine	3:7	Priolocaine	712.05	–	2.03×10^{-3}	[38, 50, 51]

Prilocaine: lidocaine	3:7	Lidocaine	762.70	−	1.42×10^{-3}	[38, 50, 51]
Paeonol:menthol (microemulsion)	4:6	Paeonol	0.30	−	−	[34]
Paeonol:menthol (microemulsion)	5:5	Paeonol	0.20	−	−	[34]
Paeonol:menthol (microemulsion)	6:4	Paeonol	0.21	−	−	[34]

2.1.2. Eutectic mixtures and other drugs

ITR is an anti-fungal compound that exerts its anti-fungal activity via inhibition of cytochrome P450, hence retarding ergosterol biosynthesis activity in the fungal membrane [46, 47]. A eutectic ITR:phenol (1:1) topical mixture prepared at room temperature was investigated for its capacity to increase ITR permeability (Figure 1) [48] in *ex vivo* mouse skin permeation, and also for use in *in vivo* deposition and irritation studies. The eutectic solution successfully improved the drug's solubility, which enhanced its permeation through the skin. This enhancement was implicated to result from the melting point depression of ITR:phenol (1:1) [49]. Adding oleic acid to the eutectic mixture further increased the permeation coefficient of ITR. This result was expected, as oleic acid, being an unsaturated free fatty acid, is frequently applied as a penetration enhancer. However, oleic acid

concentration did not affect the permeability coefficient and steady-state flux of ITR in a dose-dependent manner. In contrast, the permeation coefficient of ITR was inversely proportionate to the dose of thickening agent, hydroxypropylmethyl (HPMC) [48]. The purpose of adding HPMC is to restrict the topical solution from flowing out of the lesions. It must be noted that drug viscosity has a significant inverse influence on its diffusivity. As discussed previously, lower diffusivity causes lower steady-state flux of the drug.

An optimized eutectic based-topical formulation consisted of ITR:phenol (9%, w/w), HPMC (5.4%, w/w), oleic acid (9.0%, w/w), and benzyl alcohol (76.6%, w/w). This mixture demonstrated a permeability coefficient of 22.73 x 10^6 cm h^{-1} and a steady-state flux of 0.90 μg cm^{-2}. In the *in vivo* experiment, 49.83 μg cm^{-2} of ITR accumulated in rat skin after 12 h (Table 2). Furthermore, in the irritation test, this eutectic based-formulation did not cause any irritation to the skin of New Zealand white rabbits for up to 72 h (Figure 2). However, considering that ITR has adverse side effects on the liver (e.g. hepatotoxicity), further toxicological studies are required to affirm human health and safety.

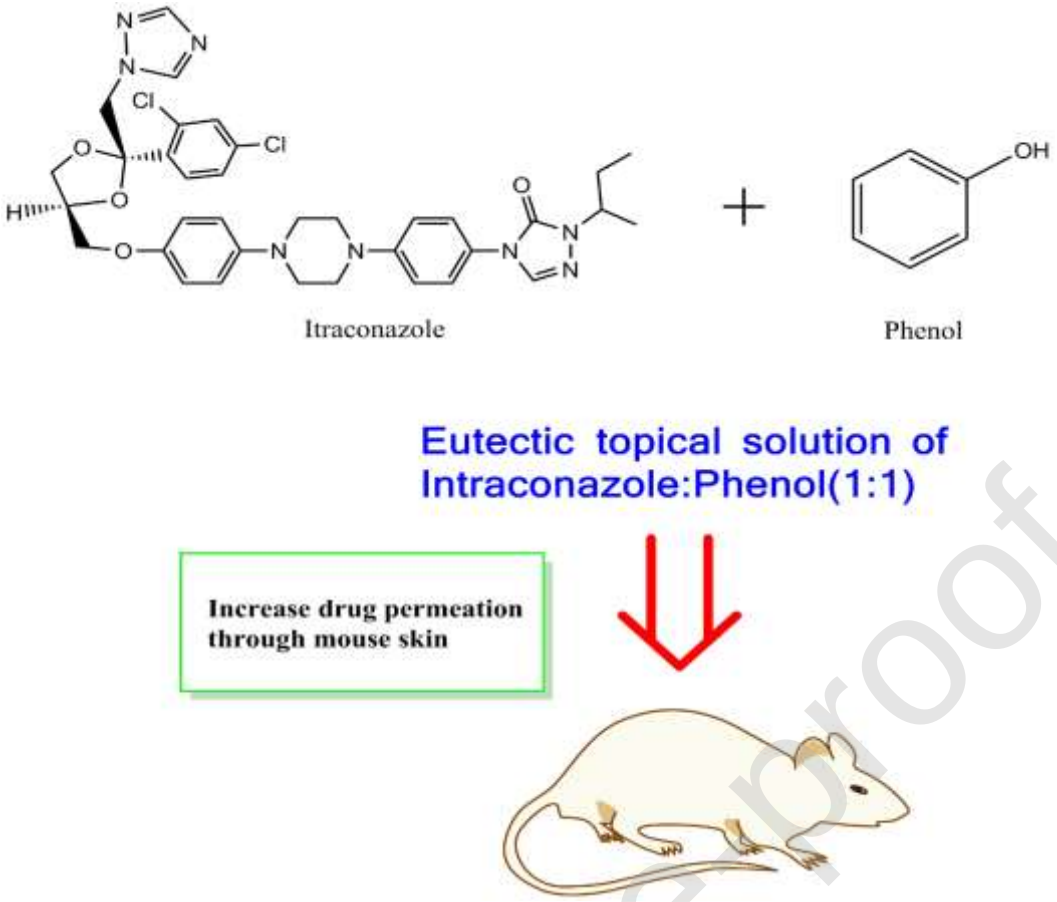


Figure 1: Eutectic topical solution of Intraconazole:Phenol (1:1).

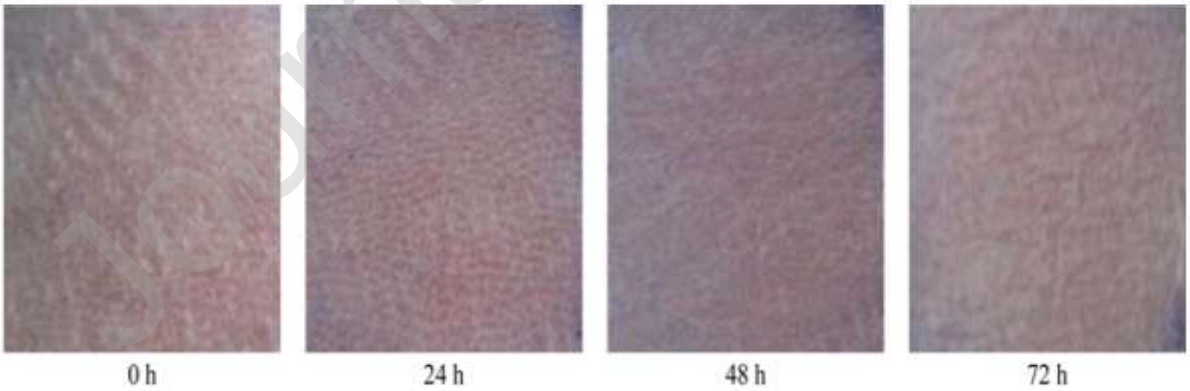


Figure 2: Images of New Zealand white rabbit skin after treatment with the optimized topical ITR-phenol eutectic solution. Based on the Draize evaluation, it scored as 0 or 1 which

indicate to minor/ no skin irritation. Reprinted with permission from Ref [52] Copyright 2012 Springer Nature.

Table 2: Distribution and accumulation of APIs in the *in vivo* studies.

DES	Ratio	API	Animal	Organ	Incubation time (h)	Amount	Ref
Choline bicarbonate: geranic acid	1:1	Insulin	Porcine	Skin	24	28.35 μg cm^{-2}	[48]
Choline bicarbonate: geranic acid	1:1	BSA	Porcine	Skin	24	9.34 μg cm^{-2}	[48]
ITR:phenol	1:1	ITR	Rat	Skin	12	49.83 μg cm^{-2}	[52]
ITR:phenol	1:1	Cannabidiol	Mouse	Abdominal skin	24	110.00 μg cm^{-2}	[47]
ITR:phenol	1:1	Cannabidiol	Mouse	Hip	24	37.43 μg cm^{-2}	[47]
ITR:phenol	1:1	Cannabidiol	Mouse	Abdominal muscle	24	11.537 μg cm^{-2}	[47]
Paeonol:ment	4:6	Paeonol	Mice	Skin	24	111.81	[34

hol (microemulsion)							]
Paeonol:menthol (microemulsion)	5:5	Paeonol	Mice	Skin	24	27.48	[34]
Paeonol:menthol (microemulsion)	6:4	Paeonol	Mice	Skin	24	28.52	[34]

Cannabidiol (CD) is a potent drug for the treatment of rheumatic diseases [50]. However, due to its high lipophilic properties, CD has low oral bioavailability, poor penetration to deeper strata, and tends to aggregate within the upper-layer membrane [51, 52]. In 2003, Lodzki et al. [50] successfully designed a eutectic mixture of CD and phosphotidylcholine to form CD ethosomes. The thermodynamic interactions between CD and phosphotidylcholine were identified by differential scanning calorimetry (DSC) and confocal laser scanning microscopy (CLSM), revealing that the eutectic mixture formed by reducing the heat of transition of phosphotidylcholine and decreasing the pre-transition of CD and phosphotidylcholine (Table 3). This eutectic system aided CD skin infusion and produced a considerable accumulation of the drug in muscle and skin. Specifically, CD accumulation was identified in abdominal skin

(110 µg cm^{-2}), hip skin, (37.43 µg cm^{-2}) and abdominal muscle (11.537 µg cm^{-2}) (Table 2). This proves that the eutecticity of CD ethosomes was able overcome the typical problems of transdermal CD delivery, such as poor penetration to deeper strata and aggregation on the upper layer membrane. In addition, the profile of CD release from the ethosomes to the plasma of male mice was examined (Figure 3). The delivery of CD reached steady-state at 24 h and was maintained until 72 h (end of experiment), and the plasma concentration of CD became constant at 0.67 µg/mL. The actual transdermal concentrations of CD after 12 h and 72 h were calculated to be 45.7 mg/kg and 86.7 mg/kg body weight, respectively. Finally, treatment with this eutectic system also ameliorated the edema and inflammation from rheumatoid arthritis in mice.

Table 3: The melting enthalpies acquired from DSC analysis. Reprinted with permission from Ref [47] Copyright 2003 Elsevier.

Sample	Energy absorbed during melting (J/g)		
	Phosphotidylcholine	CD	Eutectic mixture
Pre-transition peak	10.67	–	1.08
Transition peak	1.64	67.33	1.01

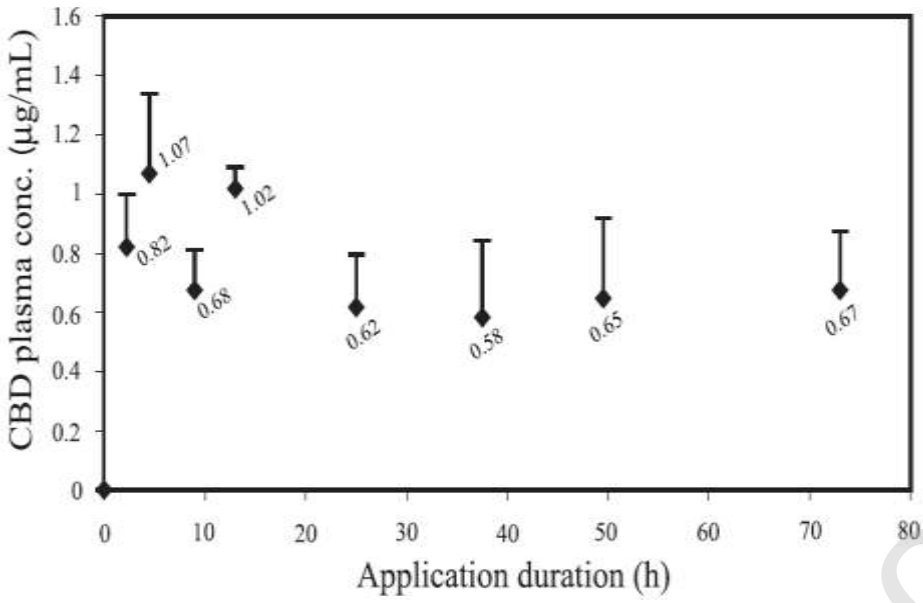


Figure 3: The profile of CD release from the CD ethosomes to the plasma of mice for 72 hours experiment. Reprinted with permission from Ref [47] Copyright 2003 Elsevier.

Protein or peptide-based drugs such as vaccines, recombinant proteins, and monoclonal antibodies have been widely studied for the prevention and treatment of many diseases such as cancer, diabetes, and infectious diseases. However, these drugs face several issues that always lead to poor patient compliance, such as short half-lives, poor cell membrane permeability, and instability (i.e. due to oxidative, proteolytic, and hydrolytic degradation) [53, 54]. In a recent study [55], a DES of choline bicarbonate and geranic acid (1:2) (CAGE) was synthesized as a novel method for the topical delivery of protein (insulin). Compared to bovine serum albumin (BSA), CAGE proved to be more efficient at transporting insulin. The transport rate and total transport of insulin using CAGE was 0.57 µg cm^{-2} h^{-1} and 28.35 µg cm^{-2}, respectively, while the transport rate and total transport of insulin by BSA was 0.19 µg cm^{-2} h^{-1} and 9.34 µg cm^{-2}, respectively. FTIR analysis showed a reduction in the peak area

relating to porcine stratum corneum lipid content, indicating lipid degradation in the stratum corneum (Figure 4). It can be postulated that CAGE improves drug permeation across the skin through the extraction of stratum corneum lipids. Compared to other formulations using PBS as a medium, CAGE also showed a significant improvement in insulin bioavailability and efficiency, with 40% reduction of blood glucose level. However, the decrease of blood glucose level observed with CAGE was lower than that achieved with microneedles/injected insulin, where a drop of 80% was observed [56]. However, unlike microneedles or injected insulin, the CAGE system demonstrated relatively sustained efficiency for a prolonged period. It can be concluded that CAGE is a suitable system for long-term glycemic treatment, and that diabetic patients are likely to have better compliance than when needing frequent injections.

Regarding CAGE, there is a fundamental question regarding its precise definition or nomenclature that remains unanswered. CAGE fits the definition of an IL, being made up of two ionic species, geranate and cholinium [57, 58]. Rogers and Gurau [59] have recently defined CAGE as an IL with complex anion [choline][geranate$_2$(H)]. However, it is also deemed a DES due to the presence of neutral geranic acid and hydrogen bonding [55]. Accordingly, Banerjee et al. [60] proposed that CAGE is entitled to all relevant classifications, including both DES and IL. Nevertheless, it is not the nomenclature but the physicochemical properties of the solvents that are most important in determining the nature and behavior of the system and its potential applications.

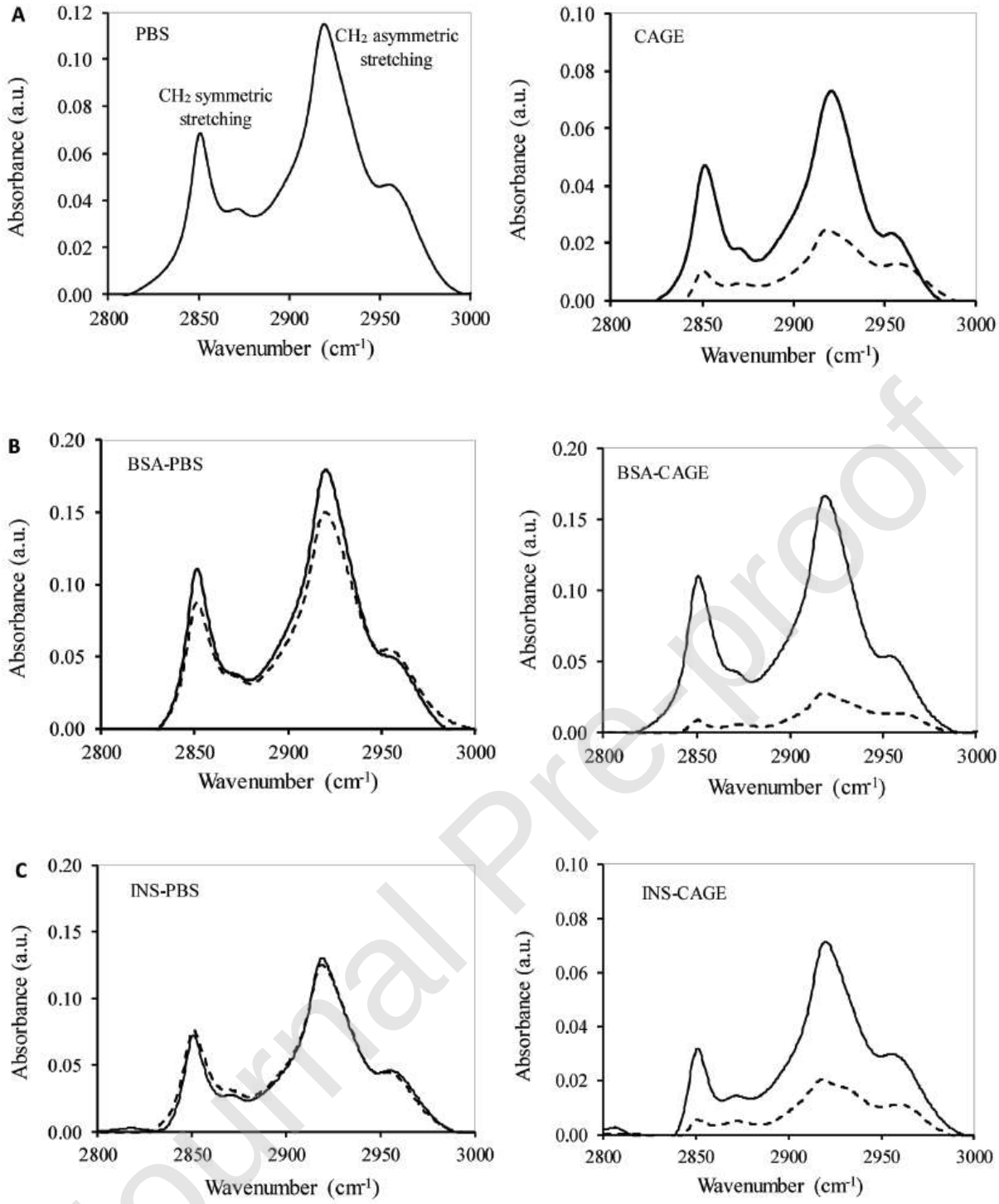


Figure 4: FTIR spectra corresponding to lipid content of the porcine stratum corneum which determined by peak area of the CH_2 asymmetric and symmetric stretching bands. Stratum corneum prior (solid black line) and post (dashed black line) application of A) PBS (solid lines and dashed are overlaid) and neat CAGE, B) BSA–PBS and BSA–CAGE, and C) INS–

PBS and INS–CAGE for 24 h. Reprinted with permission from Ref [48] Copyright 2017 John Wiley and Sons.

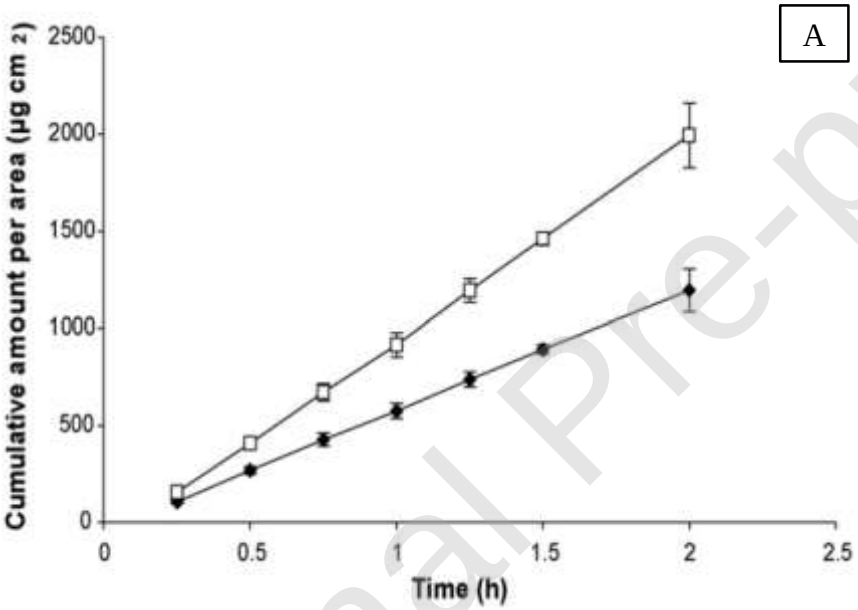
2.1.3. Dual-drug eutectic system

A promising idea in the field of pharmaceutical industry is the merging of two biological activities within one drug molecule. The role of such a dual-drug system is to confer dual biomedical/pharmaceutical functions or to provide some new synergistic active concoction that is not present with an individual drug. The penetration process of dual drugs and the effects of excipients on the skin are of great significance in transdermal drug delivery. There has been an attempt to form a eutectic mixture from a combination of two different drugs, ibuprofen (topical non-steroidal anti-inflammatory) and methyl nicotinate (topical rubefacient) [39]. This eutectic mixture (1:1) produced a single liquid phase with a melting point lower than 32 ºC (skin surface temperature) and no solid formation at skin temperature, which is in accordance with the previous report by Fiala et al. [61]. The eutectic mixture was formulated into an oil-in-water emulsion system and was found to boost permeation efficiency in an *in vitro* model of a lipophilic barrier membrane. Specifically, the mixture showed a three- to ten-fold enhancement of ibuprofen flux relative to the aqueous ibuprofen solution and the commercialized, alcohol-based ibuprofen gel formulation. This result is in agreement with a previous report [42], where the permeation flux of lidocaine and prilocaine in a eutectic mixture (lidocaine:prilocaine 49.6:50.4% w/w) increased significantly, by 2.9- to 4.1-fold and 1.8- to 2.6-fold in comparison with the respective pure lidocaine and prilocaine aqueous solutions. However, emulsified mixtures and aqueous gels of methyl nicotinate showed insignificant differences in permeation rate.

It has been shown a dual-drug eutectic system, i.e. lidocaine:prilocaine (3:7), exhibits a linear permeation profile through a silicone membrane over 2 h (Figure 5A) [61]. Figure 5B shows the relationship between the weight ratio in liquid phase and the permeation flux of eutectic lidocaine:prilocaine mixtures. Specifically, the steady-state flux of a drug is dependent on the eutectic mixture ratio, as increased proportion of an individual drug leads to an increase in its permeation. In a dual-drug system, the competition between the two drugs to penetrate the hydrophobic membrane is crucial to consider. Overall, the permeation of prilocaine through a hydrophobic silicon membrane is lower than that of lidocaine, as illustrated in Figure 5A. This result is in accordance with a previous study where prilocaine was less membrane-permeable than lidocaine, with permeability coefficients (κp) of 0.71 X 10^{-6} cms^{-1} and 1.01 X 10^{-6} cms^{-1}, respectively [62]. This proved that the mathematical prediction of κp, originally based on skin membranes, is also applicable for hydrophobic silicone membranes. It also demonstrated that the application of the eutectic solution made no significant difference to the membrane thickness, with the initial thickness being 126.14 µm and the final thickness 126.17 µm [61]. This non-significant change in membrane thickness also had no effect on drug permeation efficiency.

Another independent experiment into drug diffusivity revealed that prilocaine in a eutectic mixture has higher diffusivity than does a saturated solution of prilocaine alone, with diffusivity values of 2.03 x 10^{-3} cm^2 h^{-1} and 1.34 x 10^{-3} cm^2 h^{-1}, respectively [38, 56, 61]. In contrast, lidocaine diffusivity remained the same for a eutectic mixture and individual saturated solution, at 1.42 x 10^{-3} cm^2 h^{-1}. This proves that the steady-state flux of lidocaine is not correlated with its diffusivity behavior.

In a eutectic mixture, the membrane permeation of lidocaine is dependent on the partition process, while that of prilocaine is influenced by the diffusion process. Maximum steady-state flux was obtained when the composition had not reached equilibrium stage. This is in good agreement with a previous work [3], where the eutectic mixture that gave maximum permeation presented some solid formation in the solution (i.e. was not in the equilibrium state). Further investigation is needed in order to solve the problem of formulation instability in this eutectic system prior to application.



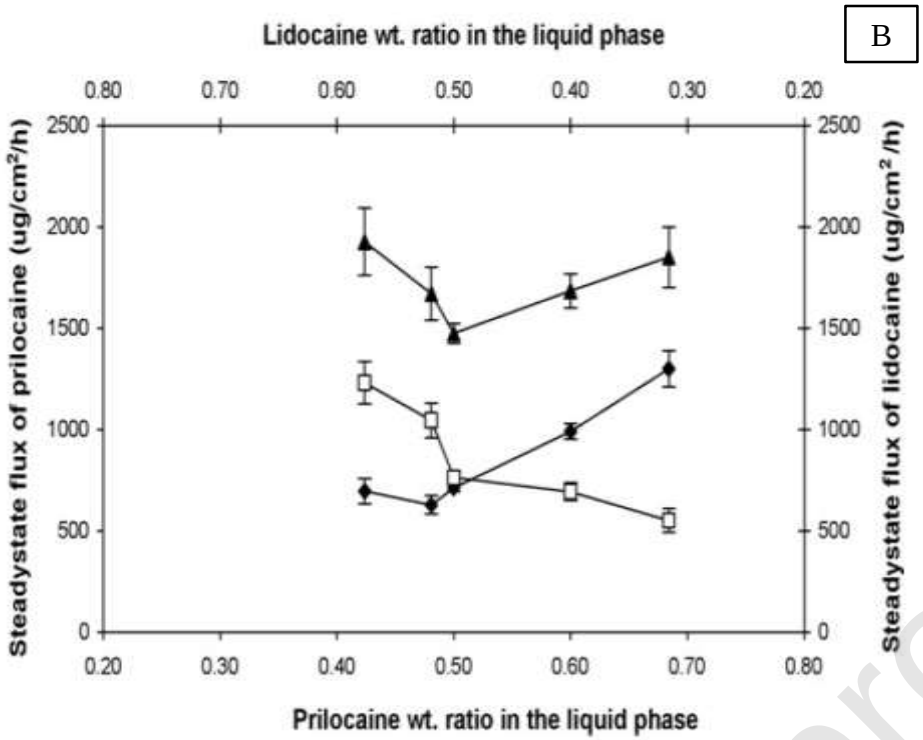


Figure 5: (A) Silicone membrane permeation profile of prilocaine and lidocaine from eutectic mixture comprising prilocaine and lidocaine at a ratio of 3:7 (B) The correlation between the weight (wt) ratio in the liquid phase of the mixture of prilocaine (♦), lidocaine (□) and total flux (Δ) and the steady-state flux of lidocaine and prilocaine from eutectic mixtures. Reprinted with permission from Ref [51] Copyright 2010 Elsevier.

2.1.4. Eutectic mixtures in microemulsions

Microemulsion formulations are capable of improving drug penetration through the skin membrane [63]. In a recent study on improving paeonol transdermal delivery, a microemulsion-based gel containing a eutectic paeonol:menthol mixture demonstrated a better drug permeation profile than did the system without microemulsion [33]. In terms of physical stability, microemulsion-based gels with paeonol:menthol at a ratio of 4:6 exhibited better stability, with no formation of needle- or rod-like crystals in the solution. By contrast,

the microemulsions with paeonol:menthol at ratios of 6:4 and 5:5 displayed some formation of needle-like, rod-like, and short bar crystals within 10 days (Figure 6). The paeonol:menthol (4:6) microemulsion system displayed the smallest droplet size and the lowest viscosity at various temperatures (i.e. 20 ºC, 30 ºC, and 40 ºC), as expected. It also exhibited the highest permeation flux (0.3 µg cm^{-2} h^{-1}) and shortest lag time (0.5 h), compared to solutions with ratios of 5:5 (permeation flux: 0.2 µg cm^{-2} h^{-1}, lag time: 0.726 h) and 6:4 (permeation flux: 0.21 µg cm^{-2} h^{-1}, lag time: 0.9 h) [33]. When tested on mouse skin, considerably greater paeonol deposition was observed for the 4:6 formulation (111.81 µg cm^{-2}) than for the other tested ratios (5:5, 27.48 µg cm^{-2}; 6:4 28.52 µg cm^{-2}). This finding is probably due to the smaller droplet size and lower viscosity of the 4:6 formulation, which enhance its fluidity and skin permeation properties. This conclusion is supported by a previous study [64] that mentioned the influence of microemulsion stability, small size, and low viscosity in improving drug release and drug penetration through skin.

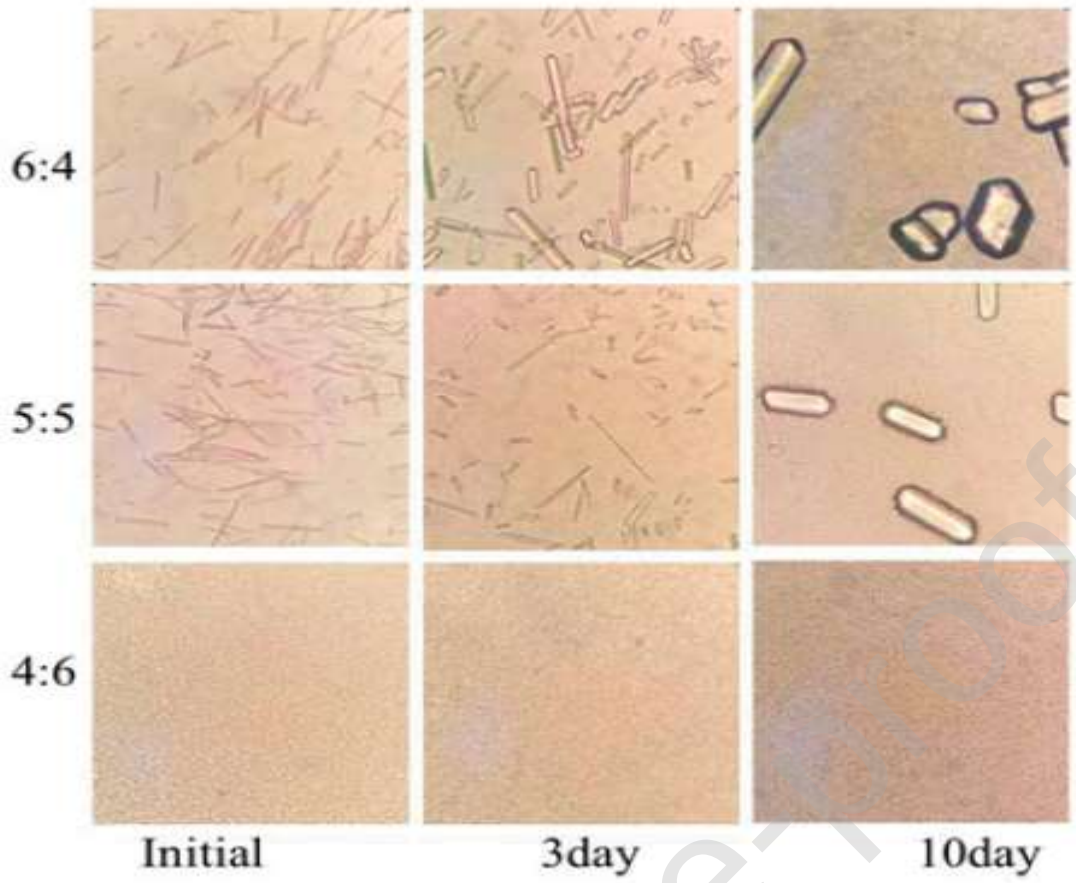


Figure 6: The micrograph of microemulsion-based gels containing eutectic mixture of paeonol:menthol in different ratio within 10 days observation (magnification 400x). Reprinted with permission from Ref [34] Copyright 2017 Taylor & Francis.

A phospholipid microemulsion-based hydrogel was prepared with a eutectic mixture of lidocaine:prilocaine (1:1) for the enhancement of topical delivery of lidocaine and prilocaine through the skin membrane (Figure 7) [65]. The optimized microemulsion assisted with n-methylpyrrolidone (NMP) ($LPME_{OPT\text{-}NMP}$ gel) demonstrated higher drug flux than did the commercial cream Prilox^{TM}, which consists of 2.5% lidocaine (w/w) and 2.5% prilocaine (w/w). This result was further supported by an *in vivo* study that showed greater efficiency for the $LPME_{OPT\text{-}NMP}$ gel (around 1.73-fold higher). This increased efficiency is probably

because of the phospholipid molecules incorporated in the system, which have the capability to alter the microenvironment of the skin being transported across. In addition, the use of a drug-based eutectic mixture in this formulation could have increased permeation efficiency due to melting point depression [44]. These attributes significantly improved the penetration and localization of drugs, as well as the interaction between drugs and skin membrane.

More generally, the combination of a drug-based eutectic system with phospholipids could maximize drug delivery efficiency. Phospholipids are known to be easily integrated into the skin membrane and to fluidize the lipid membrane matrix (Figure 8) [66], hence increasing the penetration of drugs. Phospholipids are also able to induce tissue hydration, which could assist in enhancing the percutaneous penetration of drugs [67]. Further analysis, such as study of the cell membrane by electron micrograph, is recommended in order to validate the changes induced by the phospholipid-based microemulsion system. Additionally, in a skin histology analysis, the $LPME_{OPT\text{-}NMP}$ gel resulted in less alteration of skin histology, and no irritation was observed [65]. The $LPME_{OPT\text{-}NMP}$ gel can therefore be considered as safe; however, further work is needed in order to confirm its safety at clinical stages.

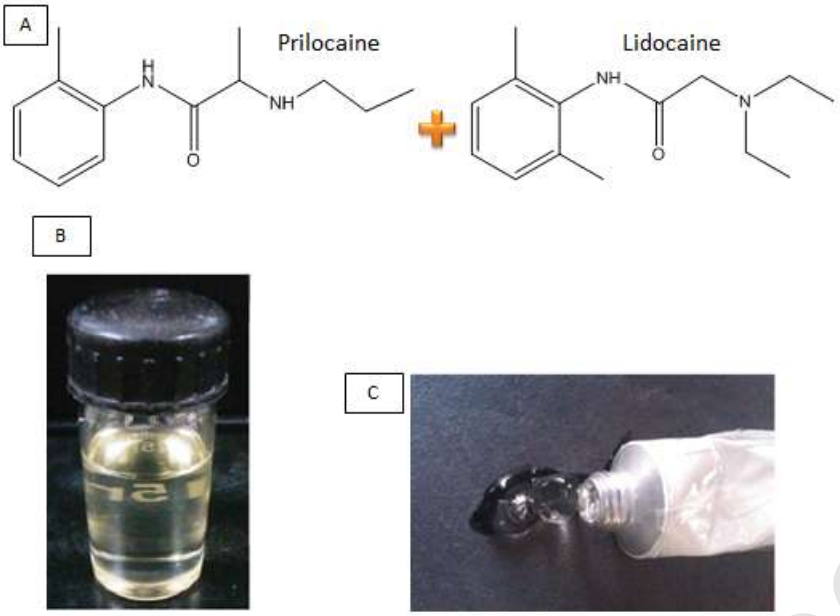


Figure 7: The preparation of $LPME_{OPT\text{-}NMP}$ gel: (A) A eutectic mixture of prilocaine:lidocaine (1:1), (B) microemulsion of prilocaine:lidocaine (1:1) and (C) $LPME_{OPT\text{-}NMP}$ gel. The photo of (B) and (C) were taken from. Reprinted with permission from Ref [67] Copyright 2016 Taylor & Francis.

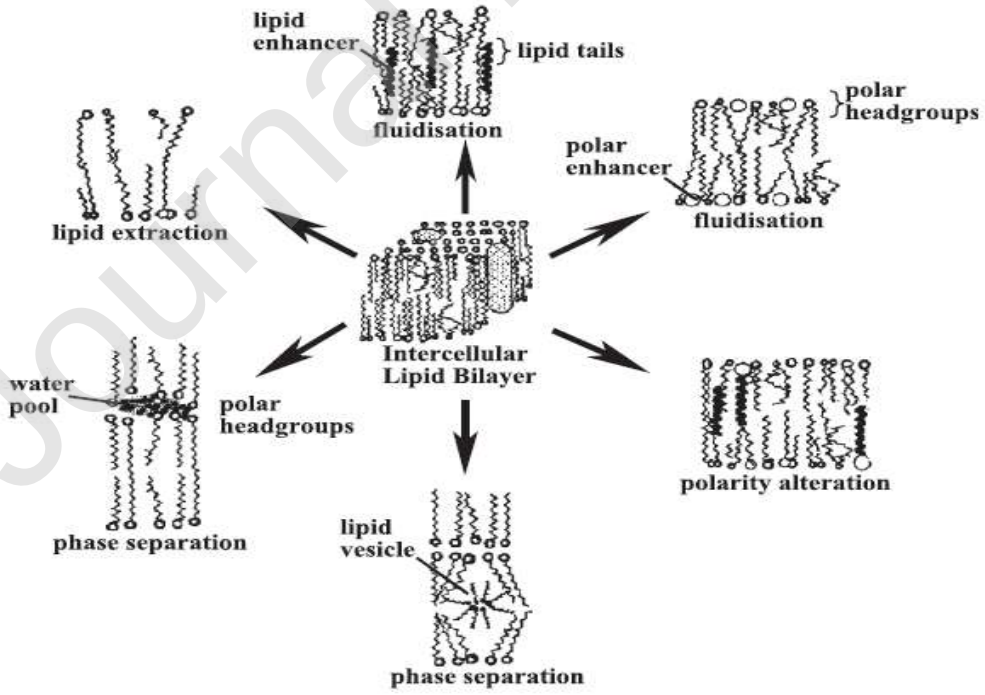


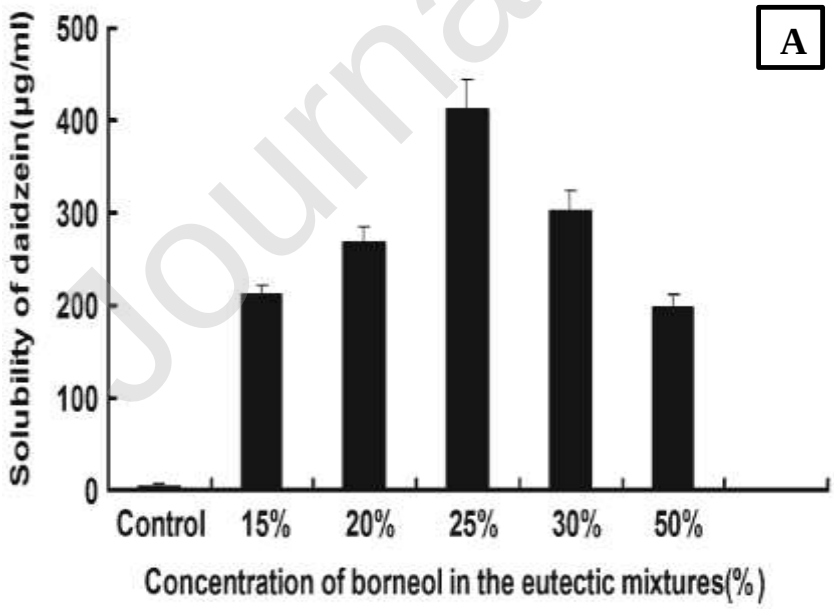
Figure 8: Mechanism of action of penetration enhancer within intracellular lipid of skin membrane, namely fluidisation, polarity alteration, phase separation and lipid extraction. Reprinted with permission from Ref [69] Copyright 2012 Elsevier.

Eutectic microemulsions have also been used as drug carriers for daidzein [68], also known as 4',7-dihyroxylisoflavone, which is a water-insoluble isoflavone and has preventive effects against colon cancer and breast cancer [69]. Unfortunately, the oral bioavailability of daidzein in rats is extremely poor [70-72]. In an attempt to increase daidzen bioavailability, its solubility was examined in microemulsions incorporating eutectic mixtures of borneol:menthol with different ratios [68]. The highest solubility (around 400 µg/mL) was observed at a borneol:menthol ratio of 1:3 (Figure 9A).

Yet another major factor influencing the efficiency of a drug is its intestinal absorption, which can be improved by the use of eutectic mixtures. A eutectic microemulsion of borneol:menthol (1:3) formulated with 6% ethyl oleate (oil), 10% PEG400 (co-surfactant), 60% water, and 20% Cremophor RH40 (surfactant) enhanced the absorption efficiency of daidzein in rat intestinal membrane in a dose-dependent manner [68]. The highest absorption occurred at 3% borneol:menthol, with a permeability coefficient (P_{app}) of almost $16x10^{-6}$ cm s^{-1} (Figure 9B). Furthermore, *in vivo* study revealed the bioavailability of daidzein was enhanced 1.5– and 3.65–fold by the microemulsion, in comparison to daidzein solution alone. In contrast, verapamil, a standard P-glycoprotein inhibitor that also acts as an excipient, showed insignificant effect on the intestinal absorption of daidzein. The enhanced absorption of daidzen when combined with the eutectic mixture is attributable to the high solubility of daidzen in borneol:menthol (1:3). However, the precise mechanism of daidzen intestinal

absorption when using this eutectic mixture-microemulsion formulation remains unclear, and merits further investigation.

Incorporation of lipid-based microemulsions in drug formulations could improve drug oral bioavailability, due to their high thermodynamic stability, high solubilization, and high drug permeation capacity [73-75]. There is probably a synergistic effect between the borneol:menthol eutectic mixture and the microemulsion that contributed to the high daidzen solubility and absorption efficiency. However, further study is needed to elucidate the interaction between the microemulsion and eutectic mixture, as well as their possible synergistic effects. Furthermore, while borneol:menthol is widely used in traditional Chinese medicine and has been considered as safe in previous reports [68, 76], further evaluation of the toxicity profile of this system is highly needed. Specifically, the microemulsion eutectic system is different from the conventional borneol:menthol mixture, and therefore may have a different toxicity profile.



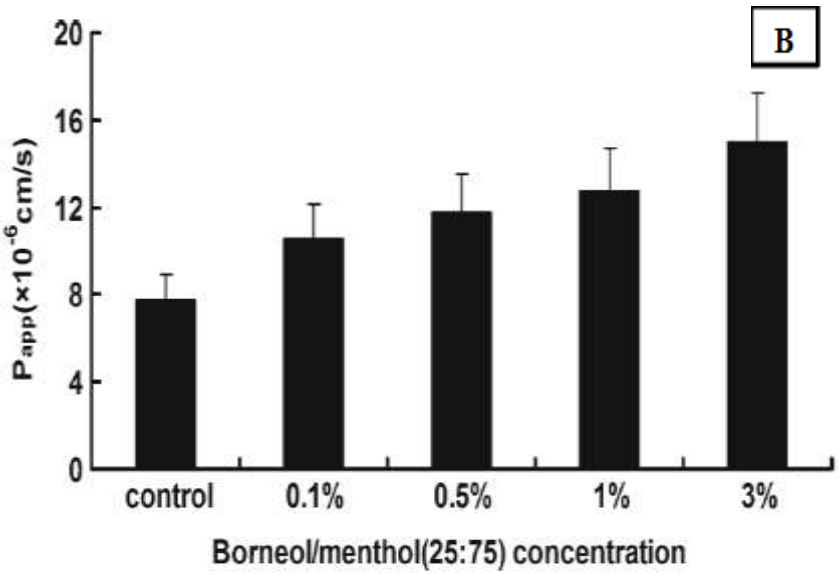


Figure 9: (A) Solubility of daidzein in different concentration of borneol in the eutectic mixture of borneol:menthol and (B) absorption efficiency daidzein in rat intestinal membrane. Reprinted with permission from Ref [46] Copyright 2011 Springer Nature.

2.1.5. Polymeric eutectic delivery system

Polymers have been incorporated with APIs to act as an excipient, sheltering them and controlling their release profiles [77]. Polymers also enhance the bioavailability and dissolution rate of drugs/APIs. However, the incorporation of drugs and polymers involves time-consuming steps such as mixing two molten substances, melt extrusion, dissolution of each substance in the solvent, and solvent exclusion [78]. Polymeric eutectic delivery systems (PES) provide a potential alternative method for the incorporation of drugs and polymers that is simpler and greener. This process also may prevent degradation of the drug's bioavailability and efficiency. The interaction between drugs and polymers in a eutectic system can occur through several mechanisms such as ionic forces and hydrogen bonding

[79]. Better molecular interaction facilitates dispersion of the solid drug in the mixture [80], thus significantly influencing the drug's permeation properties [81].

A eutectic mixture of prilocaine:lidocaine (1:3) coated with polymer poly(vinyl alcohol) (PV) and poly(acrylic acid) (PA) was prepared and its capacity to enhance both drugs permeation efficiency tested [82]. Although PA has good adhesive properties, its high viscosity may decrease the diffusion rate of an incorporated drug. Accordingly, another polymer (i.e. PV) was incorporated in order to reduce the viscosity of the mixture. Figure 10 shows that increasing the ratio of PV:PA from 1:1 to 7:1 leads to increased permeation activity for both prilocaine and lidocaine. Meanwhile, the increase of PA in the formulation resulted in increase in the mixture viscosity, thereby, the rate of drug permeation was reduced. The introduction of several additional drug permeation enhancers into the polymeric eutectic system, such as oleyl oleate, lauryl alcohol, triacetin, oleic acid and diisopropyl adipate, also resulted in no significant enhancement of permeation relative to of the control (i.e. without added enhancer) [82]. This result is not in agreement with a recent study done by Paradkar et al. [83], in which a propylene glycol and eudragit polymer was applied as a drug enhancer in a formulation with eutectic menthol:camphor (1:1) and resulted in 80% higher drug permeation activity for clotrimazole (an antifungal drug). This indicates that different combinations of polymers, drug enhancers, and eutectic mixtures may result in different drug permeation efficiencies. Furthermore, Paradkar et al. [83] showed that the optimized formulation of their polymerized clotrimazole eutectic system exhibited higher antifungal activity against *Candida albicans* (zone of inhibition 20 mm) than did either clotrimazole alone (18 mm) or the menthol:camphor (1:1) eutectic mixture alone (10 mm). This indicates that there is probably a synergistic effect between clotrimazole and the eutectic mixture of

menthol:camphor that increases the antifungal activity of clotrimazole against *Candida albicans*.

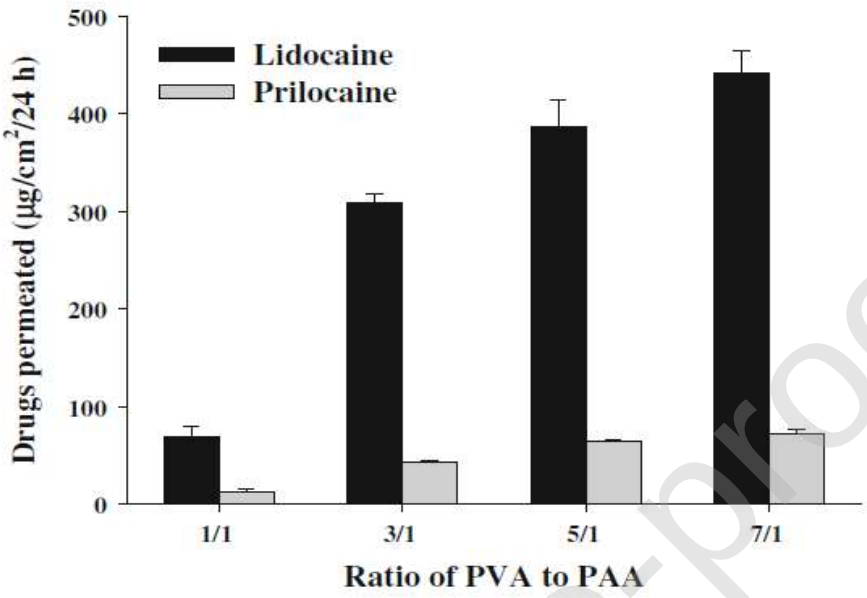


Figure 10: The permeation effect of prilocaine and lidocaine with 2.66% w/w of drug content at the ratio of 3:1 from cataplasm matrix on several ratios of PV and PA. Reprinted with permission from Ref [83] Copyright 2012 Springer Nature.

Another study using eutectic mixtures of menthol:camphor [84] identified the ratio of 5:5 to have the most optimum (low) viscosity. The system was prepared by mixing the eudragit® EPO polymer (aminoalkyl methacrylate copolymers with dimethyl aminoethyl functional group) with the eutectic mixture of menthol:camphor (5:5) to form a eudragit-based eutectic system (EES). In accordance with a previous report by Chun et al. [82], the study also showed that the viscosity of EES increased as the concentration of eudragit® EPO increased. This illustrates that polymer concentration may affect the viscosity of the mixture.

EES also displayed considerable enhancement of ibuprofen solubility, at 1.5 x 10^4 times greater than in aqueous solution. As with polymer concentration, the viscosity of the PES increased as the concentration of ibuprofen increased. The optimized EES consisting of 30% eudragit® EPO and 10% (w/w) ibuprofen in menthol:camphor (5:5) had a viscosity of 12426.75 ± 20.21 cPs and appeared to have Newtonian flow behavior. Since EES can prolong drug release for longer than seven days, such a system would be highly useful for the delivery of ibuprofen over a long period, such as in the case of periodontitis treatment. Therefore, polymeric eutectic delivery systems are a strategy of interest for maintaining drug bioavailability and also controlling drug release over a certain period of time.

2.2. Deep eutectic solvents (DESs)

2.2.1. DESs as a drug solubilization vehicle

Low solubility and dissolution issues are frequently encountered in the formulation of drugs, and numerous drug candidates fail in preclinical and clinical trials because of bioavailability and formulation problems. Thus, there is a high demand for a universal and safe drug dissolver with which to overcome these drawbacks. There is great potential in implementing DESs for this purpose due to their adjustable physicochemical properties. Table 4 shows available information on the solubility of various types of drugs/APIs in DES systems. In short, DESs are capable of dissolving a wide range of drugs/APIs, including itraconazole, lidocaine, piroxicam, benzoic acid, salvianolic acid, curcumin, danazol, and posaconazole.

Table 4: List of solubility of bioactive compounds in DESs

DES	Ratio	Bioactive compounds	Solubility (mg/mL)	Reference
ChCl:ethylene glycol	1:2	DNA	N.A	[86]
ChCl:ethylene glycol	1:2	AgCl-DNA	N.A	[87]
ChCl:glycerol	1:2	DNA	N.A	[86]
ChCl:glycerol	1:2	Salvianolic acid B	N.A	[88]
ChCl:glycolic acid	1:2	Itraconazole	6.70	[89]
ChCl:glycolic acid	1:2	Piroxicam	9.90	[89]
ChCl:glycolic acid	1:2	Lidocaine	100.60	[89]
ChCl:glycolic acid	1:2	Posaconazole	76.80	[89]
ChCl:glycolic acid	1:1.7: 0.3	Piroxicam	3.10	[89]
ChCl:glycolic acid	1:1.7: 0.3	Lidocaine	295.40	[89]
ChCl:glycolic acid	1:1.7: 0.3	Posaconazole	88.40	[89]
ChCl:glycolic acid:oxalic	1:1.7:	Itraconazole	46.40	[89]

acid	0.3			
ChCl:maleic acid	3:1	Curcumin	0.0667	[90]
ChCl:malonic acid	1:3	Benzoic acid	18.0	[91]
ChCl:malonic acid	1:3	Griseofulvin	0.0044	[91]
ChCl:malonic acid	1:3	Danazol	0.1007	[91]
ChCl:malonic acid	1:3	AMG517	0.014	[91]
ChCl:malonic acid	1:3	Itraconazole	6.60	[91]
ChCl:malonic acid	1:1	Benzoic acid	11.00	[91]
ChCl:malonic acid	1:1	Griseofulvin	0.002	[91]
ChCl:malonic acid	1:1	Danazol	0.043	[91]
ChCl:malonic acid	1:1	AMG517	0.002	[91]
ChCl:malonic acid	1:1	Itraconazole	1.20	[91]
ChCl:urea	1:3	Benzoic acid	23.00	[91]
ChCl:urea	1:3	Griseofulvin	0.0061	[91]
ChCl:urea	1:3	Danazol	0.016	[91]
ChCl:urea	1:3	AMG517	0.00022	[91]
ChCl:urea	1:3	Itraconazole	< 0.001	[91]

ChCl: urea	1:1	Benzoic acid	14.00	[91]
ChCl: urea	1:1	Griseofulvin	0.002	[91]
ChCl: urea	1:1	Danazol	0.015	[91]
ChCl: urea	1:1	AMG517	< 0.0001	[91]
ChCl: urea	1:1	Itraconazole	< 0.001	[91]
ChCl:1,2-propanediol	1:2	Aspirin	202.00	[92]
ChCl:1,2-propanediol	1:2	Acetaminophen	324.00	[92]
ChCl:1,2-propanediol	1:2	Naproxen	45.26	[92]
ChCl:levulinic acid	1:2	Ketoprofen	329.10	[92]
ChCl:proline	3:1	Rutin	2.79	[93]
Camphor:menthol	1:1	Ibuprofen	282.11	[94]
Glucose:sucrose	1:1	Curcumin	0.05211	[90]

One of the most intriguing applications of DESs as drug solubilizing vehicles is their capability to dissolve poorly water-soluble drugs/APIs. For instance, ChCl:urea (1:3) and ChCl:malonic acid (1:1) have been shown to increase the solubility of low-soluble drugs such as benzoic acid, itraconazole, griseofulvin, and also synthesized drug AMG517 by 5- to 22,000-fold over water solution [85]. Furthermore, the solubility of benzoic acid,

itraconazole, griseofulvin, and AMG517 in the DES binary mixture was considerably superior to the corresponding solubilities in individual aqueous solutions of eutectic mixture components. The study also concluded that DESs, especially ChCl:urea (1:3) and ChCl:malonic acid (1:1), are pharmaceutically acceptable for use as drug carriers in pharmacokinetic *in vivo* studies. However, it must be noted that drug dissolution is not the only parameter that needs to be considered in the pharmacology field; the toxicity profile of the drug vehicle (i.e. the DES) is another essential factor that should be taken into account.

In another work, ChCl:urea (1:2) demonstrated higher DNA solubilization with shorter length of dissolution relative to ILs [86, 87]. However, there is speculation that this DES has a denaturing effect on DNA duplexes [86, 88]. DNA dissolution capacity was also explored for new combinations of DESs, specifically comprised of ChCl with ethylene glycol, glycerol, levulinic acid or sorbitol as the hydrogen bond donor (HBD) [88]. ChCl:ethylene glycol (1:2) and ChCl:glycerol (1:2) were found to be the most efficient at dissolving DNA in concentrations of up to 2.5% and 5.5%, taking 2 and 6 h, respectively. No DNA degradation was observed by FT-IR, UV-vis, ^{31}P NMR, agarose gel electrophoresis, or circular dichroism analysis. Most interestingly, these DESs also were recyclable over three consecutive reuses. This indicates the sustainability of DES mixtures as promising multi-purpose agents.

In another study of food-borne pathogens and wound treatment [89], 2.5% w/w salmon sperm DNA was incorporated with silver chloride (AgCl) to form a poorly water-soluble anti-microbial agent. This AgCl-coated DNA was successfully solubilized in a ChCl:ethylene glycol (1:2) DES. When AgCl alone (1% w/v) was dissolved in the ChCl:ethylene glycol, hexapod formation was observed. Further addition of 2.5% w/w DNA led to the AgCl-coated

DNA taking on cephalopod- or bullet-like morphology, as shown in Figure 11. This unique morphology improved the bacteriostatic and bactericidal activity of the DNA-incorporated AgCl material against eight different species of bacteria, namely, *Escherichia coli*, *Shigella boydii*, *Shigella flexineri*, *Pseudomonas fluorescens*, *Salmonella enterica*, *Vibrio cholerae* N16961 (all Gram-negative bacteria), and *Bacillus licheniformis* and *Bacillus subtilism* (Gram-positive bacteria); the 50% inhibitory concentrations (IC_{50}) ranged between 5–103 µg/mL. However, since the health safety of heavy metal-based compounds is concern, a toxicity study of this AgCl-DNA material with respect to human health and the natural environment is highly recommended.

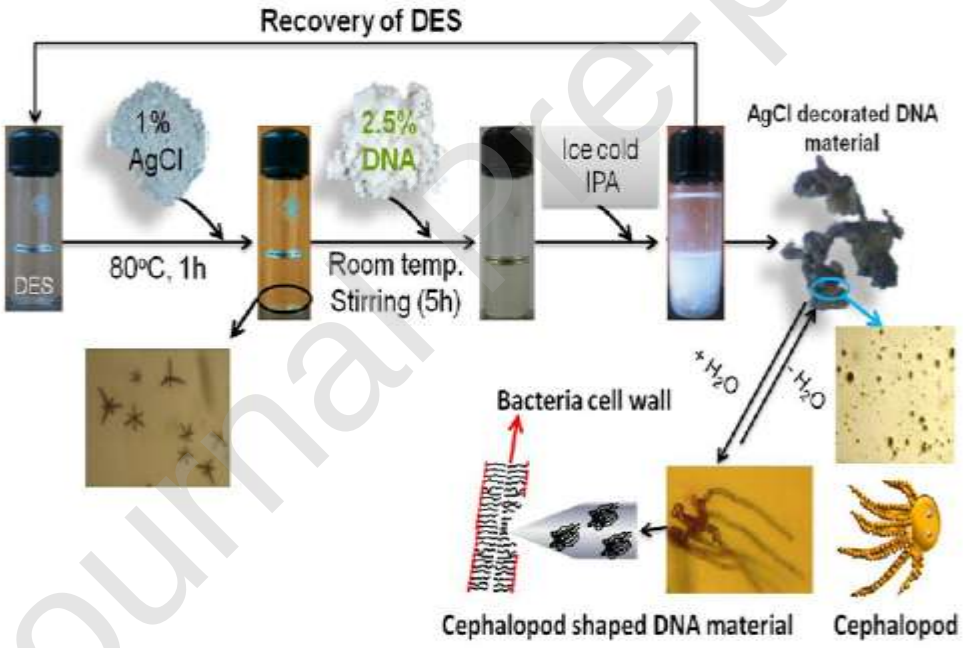


Figure 11: Illustration for the synthesis of DNA-incorporated AgCl material and its antimicrobial action. Reprinted with permission from Ref [87] Copyright 2015 Elsevier.

The occurrence of strong solvent-solute interactions between DESs and acetaminophen (ACP) was confirmed by the solubilization of acetaminophen (ACP) in various ChCl-based DESs at temperature ranging from 298.15 to 313.15 K [90], and validated by the evaluation of thermodynamic properties including volumetric and compressibility properties. The solubility of ACP in DESs increased in a dose-dependent manner positively correlated with DES concentration and temperature. This implies that the interaction between ACP and the DES was strengthened by increased DES concentration. The solubility result also was tested against the modified Yalkowsky and Apelblat, λh (Buchowski) models, of which the latter showed higher consistency with the experimental results. Among the DES formulations tested, malonic acid-based DESs showed higher solubilization than oxalic acid- and urea-based DESs. The highest solubilization was obtained for ChCl:malonic acid (1:1) at 313.15 K, with a value of 9.8765 (mole fraction) . In comparison, the solubility of ACP in a mixture of methanol (0.1 mass fraction) and water at temperature 298.15 K has been reported to be 2.65 x 10^{-3} (mole [91]. An analogous ChCl-based DES containing malonic acid (0.1 mass fraction) showed a higher solubilization of ACP at 298.15 K, 3.76 x 10^{-3} (mole fraction) . At the same mass fraction and temperature (i.e. mass fraction 0.1 and temperature 298.15 K), the solubility of ACP in ChCl:malonic acid (1:1) was also higher than that in a mixture of propylene glycol and water, with a value of 2.37 x 10^{-3} (mole fraction) [92]. Collectively, these results indicate that there the solubility of ACP is significantly improved in ChCl:malonic acid (1:1) compared to other conventional solvent systems. Meanwhile, another study [93], found aqueous DES ChCl:ethylene glycol (1:2) exhibited higher solubility of ACP than did DES ChCl:glycerol (1:2) across various mass fractions (up to 0.8 mass fraction), as illustrated in Figure 12. The solubility of ACP was dose-dependent on the mass fraction of ChCl:ethylene glycol (1:2) or ChCl:glycerol (1:2), in which increasing the mass fraction led to increased ACP solubility.

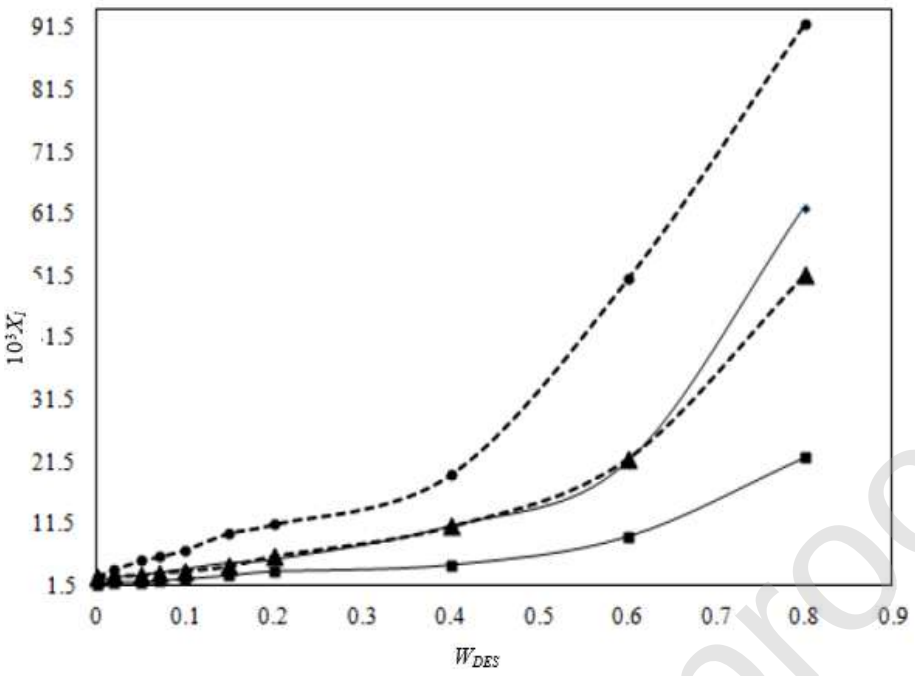


Figure 12: The solubility of ACP (mole fraction, X1) against weight fraction of DES (mass fraction, $W_{DE}S$) in aqueous solution with ChCl:glycerol at 298.15 K (■), ChCl:glycerol at 313.15 (♦), ChCl:ethylene glycol at 298.15 K (▲) and ChCl:ethylene glycol at 313.15 (●). Reprinted with permission from Ref [100] Copyright 2018 Elsevier.

Similar improvement of the solubility of ACP in DESs was also observed by Lu et al. [94]. Specifically, DESs based on ChCl, tetrapropylammonium bromide, betaine, choline bitartrate, and ethylammonium chloride all demonstrated higher solubility of ACP (i.e. 89.07–352.7 mg/mL) than water. However, the solubility of ACP was lessened as the HBD ratio increased, especially in the case of ChCl:1,2-propanediol and betaine:levulinic acid (HBD ratio range 1:2 to 1:5). In contrast, the solubility of naproxen increased with increasing HBD ratio in ChCl:levulinic acid, but decreased with increasing HBD ratio in ChCl:1,2-propanediol. Meanwhile, both ibuprofen and ketoprofen exhibited decreased solubility with increasing HBD ratio in betaine:levulinic acid but increased solubility with increasing HBD

ratio in ChCl:1,2-propanediol. It is apparent that changes in the HBD molar ratio may affect the solubility of drugs, and not all in the same way. Different drugs may interact differently with different DESs, hence influencing their solubility levels. Further study concerning the optimum HBD molar ratio is recommended in order to de-convolute this matter. In particular, while changes in HBD ratio of DESs may either increase or decrease drug solubility, the results described above encouraging pursuing this direction of research as the solubilities of tested drugs in these DESs were always higher (by 17- to 5477-fold) than their solubilities in water.

In work on curcumin compounds as photosensitizers in antimicrobial photodynamic therapy, maximum solubilization values were obtained with NADES mixtures of glucose:sucrose (1:1) (GS) and ChCl:maleic acid (3:1) (CM), at 0.05211 mg/mL and 0.0667 mg/mL, respectively [95]. For one-month storage in airtight tubes, the solubility of curcumin increased by 29% and 16% for GS and CM, respectively. Both NADESs were determined to develop no degradation side-products, as confirmed by HPLC. However, there was no significant correlation of curcumin solubility with the water content, polarity, or pH of the NADESs. NADESs are known to feature intermolecular hydrogen bonding, which is implicated in the improvement of curcumin solubilization [1, 21, 95]. The differences in solubilization between NADESs primarily arise from distinct H-bonds accepting and donating, and also the steric arrangement of the liquid crystals. In terms of solubility efficiency, the NADESs were equivalent to other solubilizers such as cyclodextrins, surfactants, and aqueous solutions containing alginate or gelatin [95, 96]. However, the GS and CM NADES exhibited better hydrolytic stability, at up to 2–10 times greater than a cyclodextrin solution and also >1300 times greater than a pH 8 solution buffer [96-98]. In addition, the GS NADES featured better photolytic stability than the cyclodextrin solution.

This result is very promising, as curcumin is susceptible to hydrolysis under alkaline conditions and photochemical reactions [98-100]. NADES of GS, GS with curcumin, and photo-assisted GS, are all considered non-toxic to *E. coli*. However, the CM NADES was toxic to the bacteria, especially at 50-fold dilution; in addition, CM with curcumin showed photo inactivation capability towards *E. coli* at a curcumin concentration of 1.25 μM [95]. The curcumin dissolved in CM possessed a higher phototoxic effect on *E. coli* than reported in previous studies [95, 101, 102]. These might be due to synergistic effects between the curcumin and CM (or NADES in general) that increase the phototoxicity (Figure 13). Specifically, photodegradation of curcumin in CM played a vital role in stimulating the production of bacteria-toxic molecules such as free radicals and reactive oxygen species. This shows that NADES has great potential for use as a photosentizer in antimicrobial photodynamic therapy.

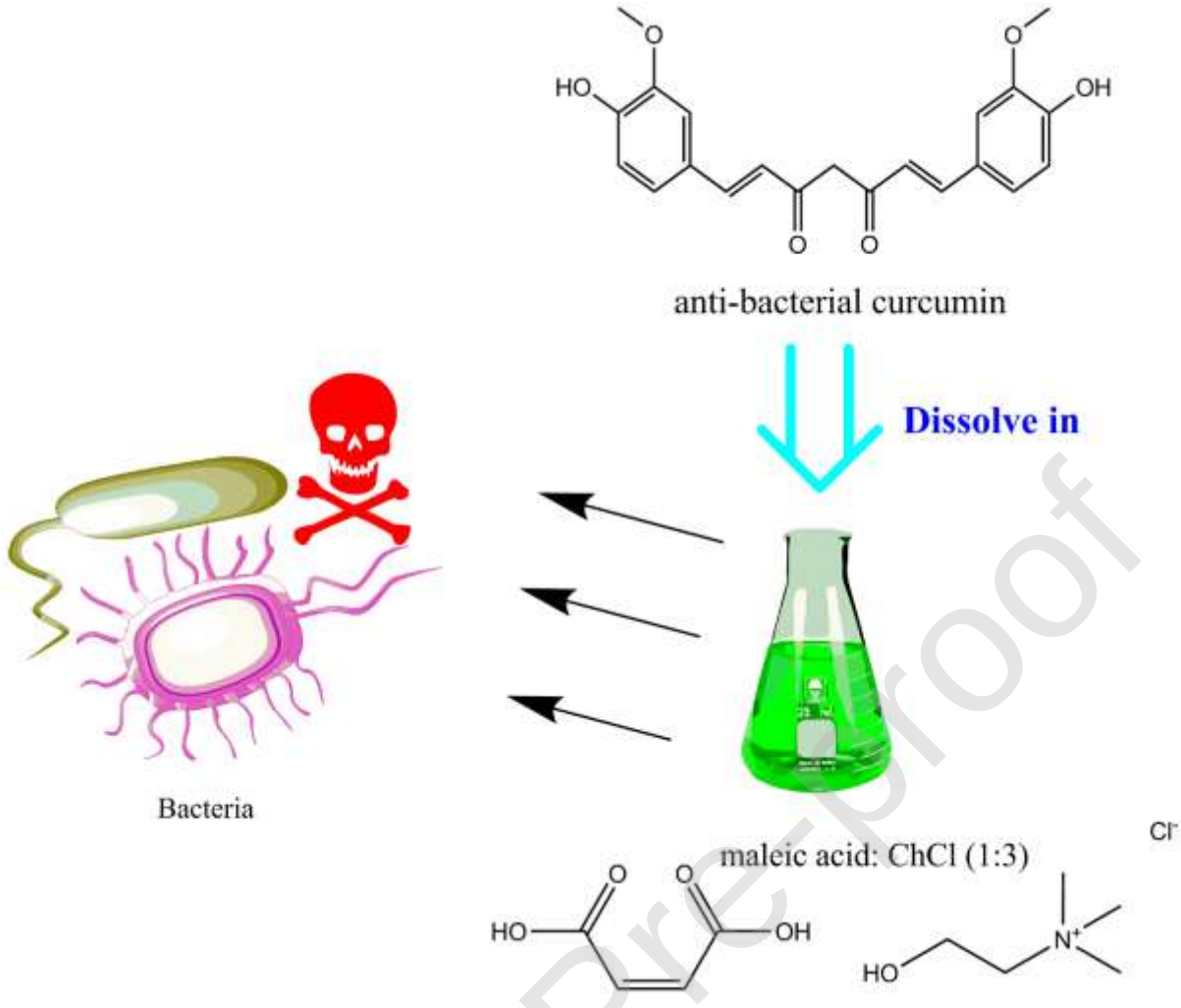


Figure 13: Anti-bacterial curcumin solubilized in NADES maleic acid: ChCl (1:3) inhibits bacteria growth.

Another study evaluated various combinations of natural components such as sugars, amino acids and organic acids for their effects on NADESs-solubilized berberine [103]. However, among the thirty-eight types of NADESs tested, the majority showed lower berberine solubility at 22 ºC compared to ethanol (2.75 mg/mL) or water (2.10 mg/mL). For instance, proline:ChCl in various ratios (i.e. 1:1, 2:1, and 1:2) was inefficient at dissolving berberine, demonstrating low solubility and stability. In contrast, a tailor-made NADES of proline:malic acid:lactic acid:water (1:0.2:0.3:0.50) exhibited the highest berberine solubility, at 25.0

mg/mL. An earlier study [104] reported that malic acid had a good interaction with berberine, resulting in the formation of soluble berberine malate. This can explain the enhancement of berberine solubility in this quaternary NADES [25, 105]. Sut et al. [103] concluded that a NADES tailor-made for the solubilization of berberine must incorporate a combination of amino acids, organic acids (malic or lactic), and water. The addition of an appropriate proportion of water to DESs or NADESs may significantly change their physicochemical properties, which subsequently influence drug solubility.

In a work on drug solubility enhancement using DESD, the solubility of itraconazole in a ternary DESD ChCl:glycolic acid:oxalic acid (1:1.6:0.4) was found to be markedly higher than in aqueous solution (5.36 mg/mL, a 53,600-fold increase) [29]. The solubilities of other tested drugs lidocaine, posaconazole, piroxicam, and itraconazole were also increased by 28-, 640-, 430-, and 6700-fold relative to the corresponding solubility in aqueous solution. Furthermore, itraconazole, lidocaine, and posaconazole displayed higher solubility in the ternary DESD system of ChCl:glycolic acid:oxalic acid (1: 1.7: 0.3), with respective solubilities of 46.4 mg/mL, 295.4 mg/mL, and 88.4 mg/mL, than in the binary DESD ChCl:glycolic acid (1:2), with respective solubilities of 6.7 mg/mL, 100.6 mg/mL, and 88.4 mg/mL (Table 4). This improved drug solubilization is mainly ascribed to polarity alteration of the basic drug in the acidic DESD through a protonating mechanism. For instance, the carboxylic acids used in a DESD can provide an adequate source of free-flowing protons to cause polarity alteration of the basic drug, hence increasing the drug's solubility [29]. The proton ionization of a DES is explained by the Hammett acidity formula (Eq. 1) [106-109].

$$H_0 = pK(1)aq + \log\left(\frac{[I]_s}{[IH^+]_s}\right) \quad (1)$$

Where pK(1)aq is the pKa value in the aqueous solution, $[I]_s$ is the molar concentration of unprotonated molecules in the solution, and $[IH^+]$ is the molar concentration of protonated molecules.

The outstanding improvement in drug solubility observed with DESDs proves the applicability of DESs and their derivatives in improving drug delivery efficiency. Therefore, it is essential to look for tangible and intangible means for maximizing the usefulness of drugs and their delivery. Future investigations should be conducted in a systematic way in order to obtain expected results with no further delay. This can be achieved through integrative endeavors of the scientific community.

2.2.2. THEDESs

Currently, the development of drug-based or active pharmaceutical ingredient (API)-based DESs that boost the pharmacological performance of the API has attracted increasing interest [78, 110]. As introduced in Section 1.0, the term THEDES is widely used for DESs that contain an API as part of their compositions [31]. Notably, the thermal properties of THEDES have been found to be different than those of their starting constituents [32]. As shown in Figure 14A, menthol demonstrated two melting points (T_m), 29 and 35 ºC, and the crystallization point was 16 ºC. Meanwhile, the thermogram of ibuprofen showed its T_m at 77 ºC and glass transition temperature (T_g) at –41 ºC (Figure 14B). However, only one thermal event was evident in the thermogram of THEDES menthol:ibuprofen (3:1), the T_g at –50.1 ºC (Figure 14C). The prepared THEDES was a stable amorphous compound and remained stable after the sequential thermal treatment cycles. The difference in the thermal behavior of the THEDES is evidence of the interaction between ibuprofen and menthol that forms the THEDES. Another study evaluating THEDES of menthol:acetylsalicylic acid (3:1) and

menthol:phenylacetic acid (1:1, 2:1, and 3:1) documented changes in their carbonyl groups as a result of intermolecular interactions between the carbonyl groups of the acetylsalicylic acid and phenylacetic acid with the hydroxyl group of the menthol [31]. These analyses proved that interactions occur between DES components and APIs to form a THEDES. In a more recent study [111], NMR analysis confirmed the formation of a THEDES through hydrogen bonding between menthol and the API (benzoic acid, ibuprofen, and phenylacetic acid). Pulsed-field gradient (PFG) NMR analysis indicated that the diffusion mechanism of each component of the THEDESs was similar to that observed in ILs and ChCl-based NADESs, in which a species migrates by jumping between holes or voids in the mixture generated via punctual thermal fluctuations [111, 112].

THEDESs development has drawn intense attention from researchers, especially in the fields of biomedicine and pharmacy. The use of THEDESs in drug delivery applications has been reported to boost efficacy in terms of drug solubility, bioavailability, and skin permeation [31, 32, 111]. A list of THEDESs and their applications in drug delivery is shown in Table 5. The application of THEDESs to drug dissolution enhancement and drug permeation will be further discussed in the following Sections 2.2.3 and 2.3.

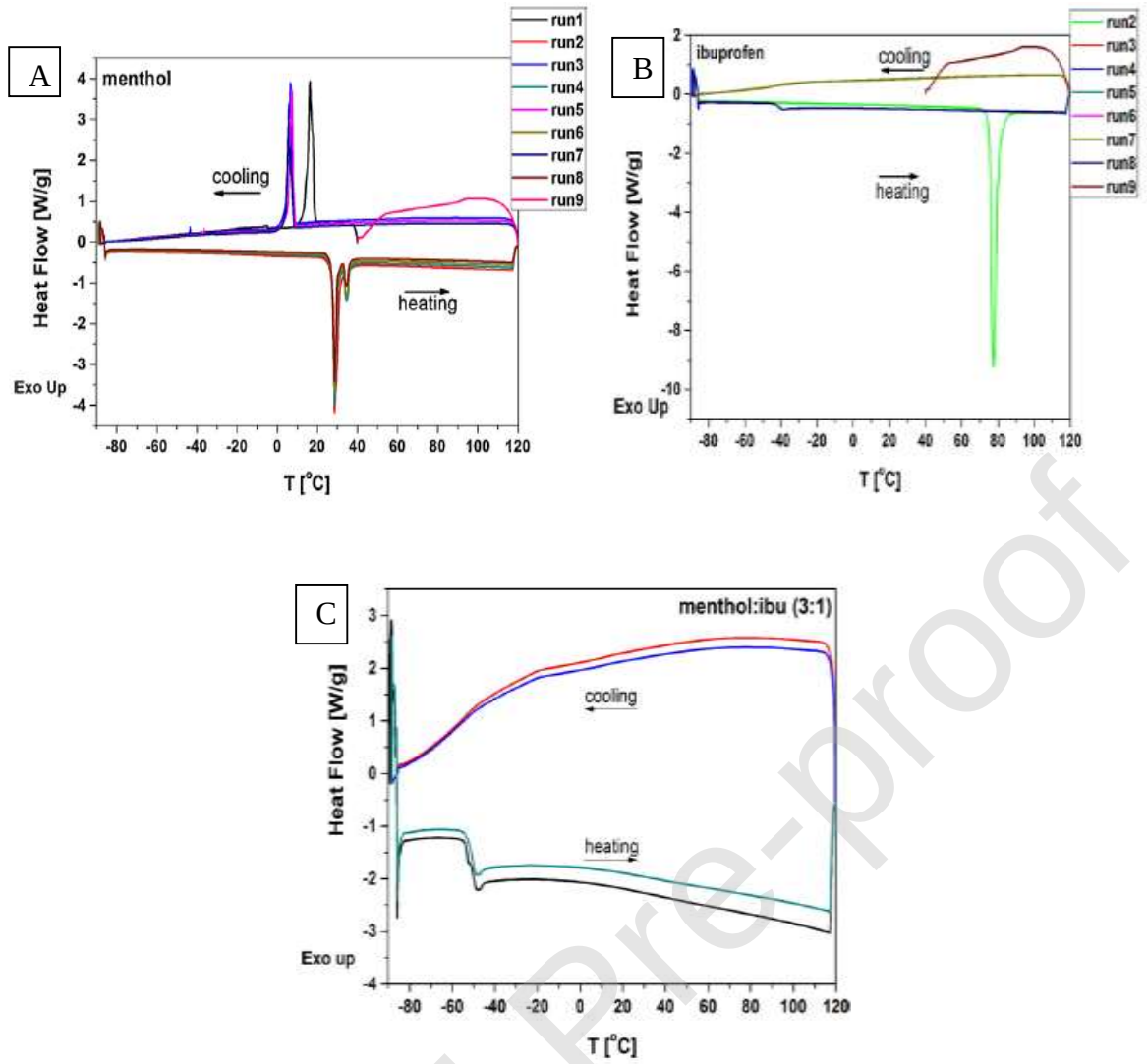


Figure 14: (A) DSC thermogram of racemic menthol, α and β, (B) ibuprofen (C) THEDES menthol:ibuprofen (3:1). Reprinted with permission from Ref [32] Copyright 2015 Elsevier.

Table 5: List of therapeutic deep eutectic solvent (THEDES)

THEDES	Ratio	Application	References
Cannabidiol:phosphotidylcholine	N.A	• Enhance transdermal delivery • Better accumulation in muscle and skin • Ameliorate edema and	[47]

		inflammation	
Ibuprofen:1,8-cineole	2:3	• Enhance transdermal delivery	[3]
Ibuprofen:LD-menthol	1:3	• Enhance transdermal delivery	[3]
Ibuprofen:L-menthol	3:7	• Enhance transdermal delivery	[3]
Ibuprofen:L-menthol	1:1	• Enhance transdermal delivery	[3]
Ibuprofen:thymol	2:3	• Enhance transdermal delivery	[3]
Lidocaine:prilocaine	3:7	• Enhance transdermal delivery	[51]
Menthol:phenylacetic acid	2:1	• Anti-microbial agent	[31, 32]
Menthol:phenylacetic acid	3:1	• Anti-microbial agent	[31, 32]
Menthol:benzoic acid	3:1	• Anti-microbial agent	[31]
Methyl nicotinate:ibuprofen	1:1	• Enhance transdermal delivery	[39]
Capric acid:menthol	4:1, 1:1, 1:4	• Enhance drug solubility	[117]

2.2.3. Drug dissolution enhancement via THEDESs

API-containing THEDES have different dissolutions than does the API alone, because they are in a different form. For example, an ibuprofen-based THEDES exists in liquid form while isolated ibuprofen exists in powder form. As mentioned previously [113], the physicochemical properties of the API may influence its dissolution. Early work revealed that the dissolution efficiency of ibuprofen released from a THEDES of ibuprofen:menthol (1:3)

was similar to that of pure ibuprofen [32]. A more recent study additionally reported that the dissolution of ibuprofen from ibuprofen:menthol (1:3) THEDES was up to 12-fold greater than that of ibuprofen alone [111]. The release of ibuprofen by the THEDES was speculated to result from disruption in the interaction between ibuprofen and menthol when the THEDES was dissolved in phosphate-buffered saline (PBS) solution [32]. Dissolution of ibuprofen increased significantly when the ibuprofen:menthol (1:3) THEDES was mixed with a starch:poly-ε-caprolactone (SPCL) (3:7) mixture by supercritical fluid sintering (Figure 15). This might be due to the unique morphology of the SPCL matrix co loaded with ibuprofen:menthol; namely, it has higher porosity and interconnectivity, which contributes to better diffusion of the drug from the matrix to the media [32, 114]. All told, these results are in accordance with other APIs that displayed higher dissolution efficiency when incorporated into a THEDES, with particularly notable results being obtained from menthol-based THEDES such as menthol:acetyl salicylic acid (3:1) (72%), menthol:benzoic acid (3:1) (87%), menthol:phenyl acetic acid (2:1) (78%), and menthol:phenylacetic acid (3:1) (81%) [31]. By contrast, dissolution efficiencies of the ChCl-based THEDES ChCl:acetylsalicylic acid (1:1) and ChCl:phenylacetic acid (1:1) were only 21% and 67%, respectively. Apparently, changing the hydrogen bond acceptor from ChCl to menthol significantly improves the dissolution efficiency.

THEDESs incorporating menthol and one of three different anti-microbial APIs, namely phenylacetic acid (PA), benzoic acid (BA), and acetylsalicylic acid (AA), all showed higher solubility in PBS solution than the corresponding API in powder form. The final THEDESs of menthol:PA (3:1), menthol:PA (2:1), and menthol:BA (3:1) all demonstrated high dissolution efficiency (i.e. 81%, 78%, and 87%), and were observed to be liquid at room temperature [31]. It is worth noting that ratio is influential on the stability of the THEDES.

THEDES menthol:BA at ratios of 1:1 and 2:1 and menthol:PA at a ratio of 1:1 all formed crystals at room temperature, as observed by polarized optical microscopy analysis. However, as the ratio of menthol in either mixture increased to 3:1, the resulting THEDES was less crystallized. It is interesting to note that homogenous THEDESs (with no crystal formation) were stable for at least two months. This level of stability is paramount for the development and design of a safe and effective drug carrier/medium.

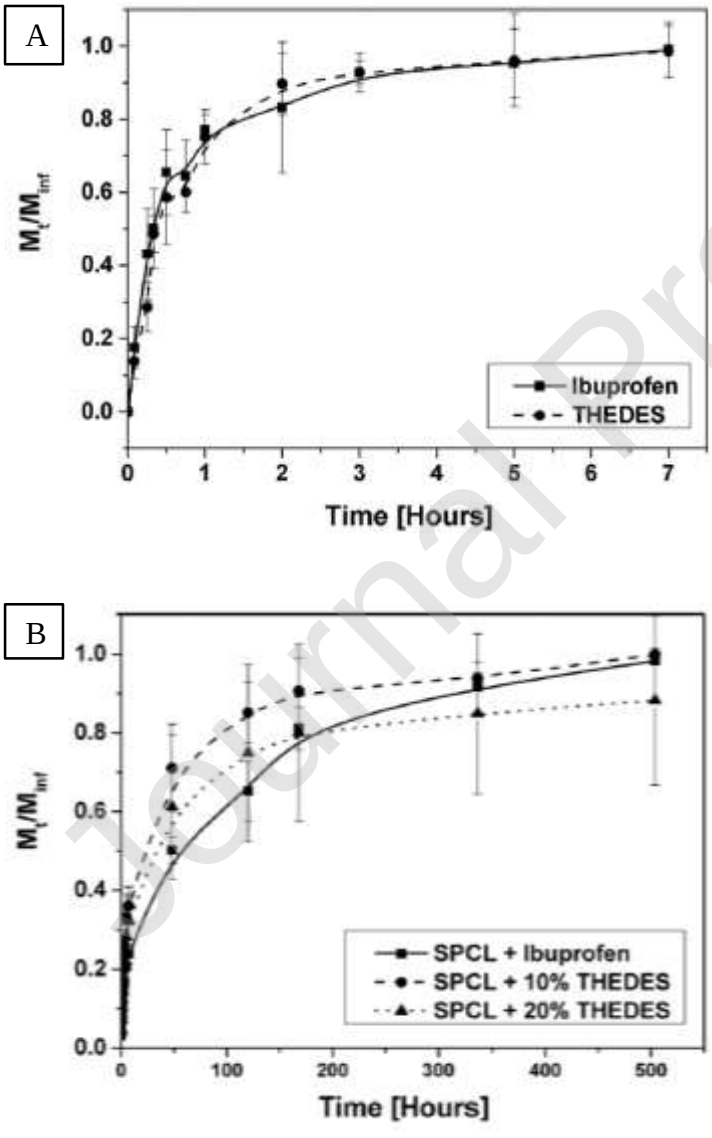


Figure 15: Comparison of dissolution profile of ibuprofen from (A) THEDES of ibuprofen:menthol (1:3) and (B) SPCL incorporated with THEDES. Reprinted with permission from Ref [32] Copyright 2015 Elsevier.

2.3. Drug permeation

2.3.1. Ibuprofen-based THEDESs

Most reported THEDESs were used to enhance transdermal drug delivery (Table 6). Among the drugs evaluated in THEDESs applications, ibuprofen was the most-studied; other types of drugs remain poorly explored. A recent study [111] investigated the relationship between ibuprofen solubility in THEDES and its permeability through a membrane, and found that ibuprofen permeability increased proportionately with ibuprofen solubility. A THEDES of menthol:ibuprofen (3:1) showed three-fold increment in permeability through a polyethersulphone membrane (14 x 10^{-5} cm s^{-1}), relative to ibuprofen alone (4.6 x 10^{-5} cm s^{-1}) (Table 6). A drug with permeability greater than 6 x 10^{-6} cm s^{-1} is considered highly permeable according to Duarte et al. [111]. Powder ibuprofen was originally categorized as Class II in the biopharmaceutical classification system [115]; THEDES menthol:ibuprofen (3:1) could be counted as Class I due to the improvements in solubility and permeability (Figure 16). However, existing studies used a synthetic membrane, and it is recommended to use a mammalian skin membrane for a better and more realisitic understanding of the interactions and conditions involved in the transdermal delivery system.

Table 6: Permeability of API and THEDES systems. Reprinted with permission from Ref [49] Copyright 2017 Elsevier.

APIs/THEDESs	Permeability (10^5 cm s^{-1})
Ibuprofen	4.6±0.14
Ibuprofen: menthol (1:3)	14.0±1.53
Benzoic acid	0.9±0.01
Benzoic acid: menthol (1:3)	6.8±0.63
Phenylacetic acid	16.0±2.30
Phenylacetic acid: menthol (1:2)	18.0±0.38
phenylacetic acid: menthol (1:3)	13.0±0.59

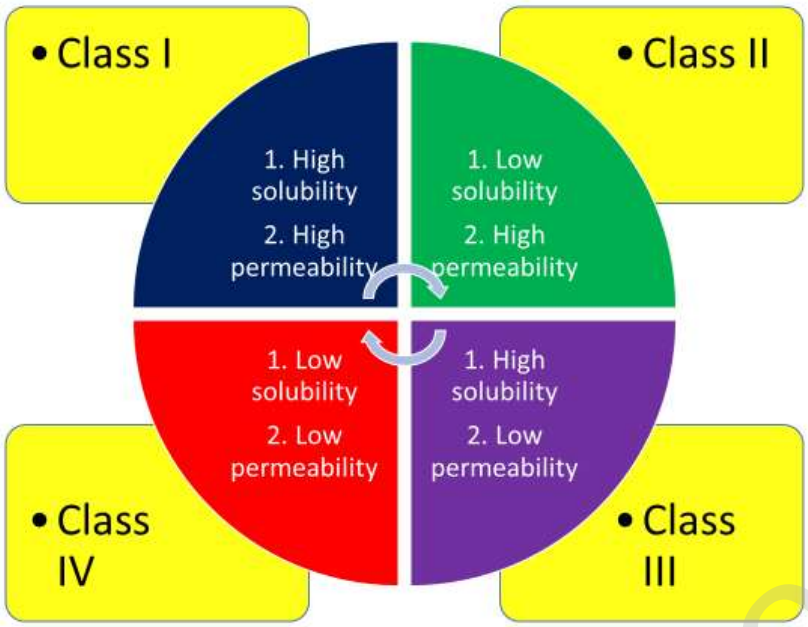
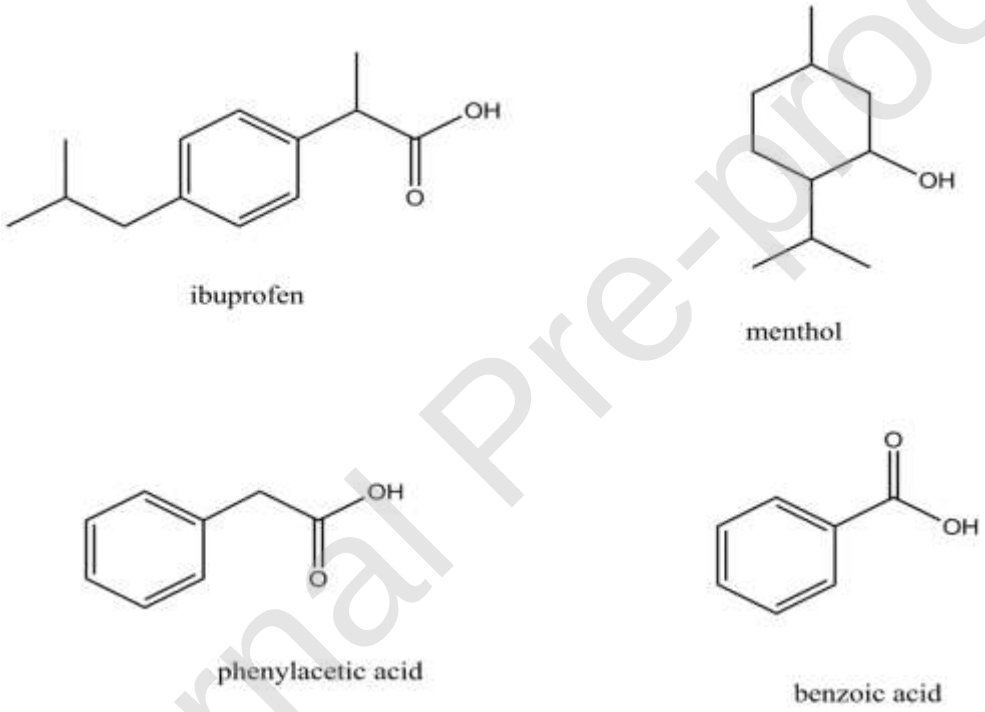


Figure 16: Biopharmaceutical characterization system of drugs.

2.3.2. THEDESs from other drugs

Phenolic acids such as phenylacetic acid and benzoic acid have anti-microbial activities against a wide range of microbial strains [31, 32, 116, 117]. Duarte et al. [111] has investigated the permeability enhancement of these APIs using THEDES formulations (Scheme 2). Of tested THEDES, phenylacetic acid:menthol (1:2) presented the highest rate of permeability (18 x 10^5 cm s^{-1}), surpassing both THEDES benzoic acid:menthol (1:3) and THEDES ibuprofen:menthol (1:3). However, this result was not significantly different from that of individual phenylacetic acid (Table 6). Meanwhile, significant improvement of API permeability was found for THEDES benzoic acid:menthol (1:3), at an increment of 7.5-fold increment (i.e. 6.8 x 10^5 cm s^{-1} compared to 0.9 x 10^5 cm s^{-1} for benzoic acid alone).

In terms of solubility, THEDES benzoic acid:menthol (1:3) demonstrated higher solubility (218.7 mg/mL) in PBS solution than did the other THEDESs and APIs tested (all <110 mg/mL). This greater solubility could be the reason for the higher driving force and penetration into the membrane observed for this formulation. Benzoic acid is categorized as a Class III drug; in this THEDES formulation, it could be promoted to Class I (Figure 16). However, again, further study using mammalian skin membrane is suggested to better represent the transdermal delivery system.



Scheme 2: Components used for the preparation of the THEDESs done by Duarte et al. [49].

2.3.3. Polymerized drug-based DESs

After polymeric eutectic mixture were introduced, polymerized drug–based DESs began to emerge as an alternative to and expansion of efforts in the development of drug transdermal

delivery systems. Frontal polymerization of DES systems has been introduced as a way to improve drug transdermal permeation [78, 110, 118]. One study prepared two DESs consisting of 3:1 mixtures of methacrylic acid (MAA) or acrylic acid (AC) (acting as HBDs) with lidocaine hydrochloride (LidHCl) (as ammonium salt). These ready-to-polymerize mixtures were then polymerized via free-radical frontal polymerization. During this process, hydrogen bonding between the DES components plays a vital role in maintaining API stability against high-temperature denaturation [119]. Both polymerized DESs displayed a homogenous and solid monolith with no segregation of LidHCl. Analysis of FTIR spectra demonstrated a total disappearance of monomers in the polymerized-DES complex, indicating complete polymerization (Figure 17).

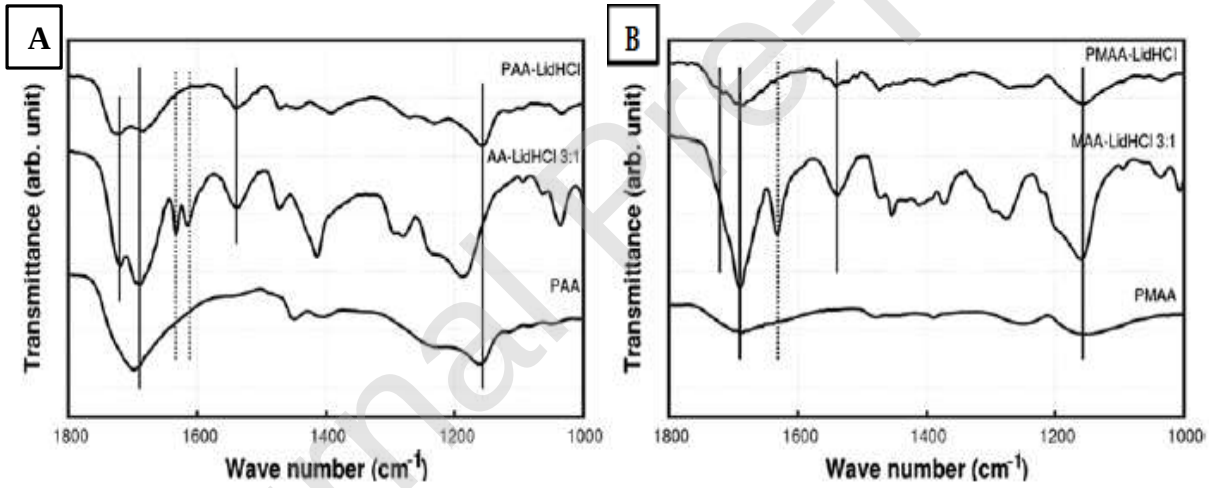


Figure 17: FTIR spectra of pure polymers, LidHCl-based DESs and the polymerized LidHCl-based DESs. As several important peaks are marked with solid lines while the monomer peaks are marked with dashed lines. Figure (A) for polymer PAC and (B) for polymer PMAA. Reprinted with permission from Ref [79] Copyright 2013 Royal Society of Chemistry.

Both polymer-based DES systems confer the possibility of a controlled, stimuli-responsive release of LidHCl (e.g. through pH and temperature). LidHCl is an API whose degree of dissolution, ionization, and delivery are pH dependent; an increase of the medium pH leads to the alteration of positively-charged molecules to electrically-neutral species. The ionization of LidHCl (pKα = 7.16) at pH 6 is described by the following Eqs.:

$$LidHCl + H_2O \leftrightarrow LidH^+ + Cl^- \quad (2)$$

$$LidH^+ \leftrightarrow Lid + H^+ (pK\alpha = 7.16) \quad (3)$$

Hence lowering the solubility of the API in an aqueous mixture and eventually causing it to be precipitated out from the solution [120]. At every pH and ionic strength condition tested, the kinetics of LidHCl release from poly(methacrylic acid) (PMAA) and poly(acrylic acid) (PAC) DES systems were in the maximum theoretical range [78]. Proton nuclear magnetic resonance (^{1}H NMR) spectra attested that LidHCl was the only compound released from the system, and its structure was maintained (not denatured). Therefore, the chance of losing LidHCl efficiency in these DES systems is expected to be low.

The optimum temperature for the frontal polymerization of DESs was at 80–120 °C, with complete conversion of monomers to polymers (i.e. from MAA and AC to PMAA and PAC) [118]. In the context of frontal polymerization, this temperature range is considered mild [110]. Importantly, complete polymerization at a mild temperature is paramount in order to easily control the drug's release and also to avoid the release of potentially toxic by-products.

Another method of releasing a drug from a drug-incorporated polymer is by applying ultrasound to the system [121]. Ultrasonic drug delivery has been widely used for drug delivery from micelles and copolymers [122], and this system is effective in treating tumors

in vitro and *in vivo* [122-125]. Therefore, it would be interesting to evaluate ultrasonic drug delivery for application to drug release from polymerized drug-based DESs.

Another study utilized a DES as a synthesis medium, with it providing essential ingredients for API synthesis. In this work, the lidocaine drug carrier poly(octanediol-*co*-citrate) elastomers (POS) was synthesized using DESs comprised of 1,8-octanediol and lidocaine [126]. A number of ratios were prepared (i.e. 1:1, 1:2, and 1:3) and were mixed at 80 ºC for 2 h. As the synthesis medium, DES played a vital role in solubilizing citric acid at temperatures below 100 ºC and also assisted in incorporating lidocaine with POS constituents (Figure 18). The optimized POS (1,8-octanediol:lidocaine at ratio 1:3) possessed high loading of lidocaine and was able to maintain the lidocaine structure, as validated by ^{1}H NMR analysis. However, further research is highly recommended to evaluate the effect of POS on drug permeation and delivery.

In a recent work [127], a gelatin-based THEDES was tested for the development of a fast-dissolving delivery system. The THEDES of ChCl:mandelic acid (1:2) encapsulated in a gelatin membrane was observed to rapidly dissolve in PBS solution and also showed no cytotoxicity effects on a mammalian cell line. This system also was found to retain the antibacterial properties of mandelic acid against both Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*). While these results are promising, further investigation of pharmacokinetic parameters such as drug release profile are essential in order to validate the superiority of this system over other conventional drug delivery systems.

Overall, the use of polymerized drug-based DESs for drug delivery applications employs three modalities of action—namely, acting as an essential monomer for the preparation of an elastomer, acting as synthesis media for polymerization, and enhancing drug release efficiency. This suggests that polymerized drug-based DESs have great potential for the development of novel drug delivery systems. However, more investigation is required, especially regarding comparison studies with other drug delivery systems (e.g. conventional polymer-based drug delivery systems) and also *in vivo* biological studies.

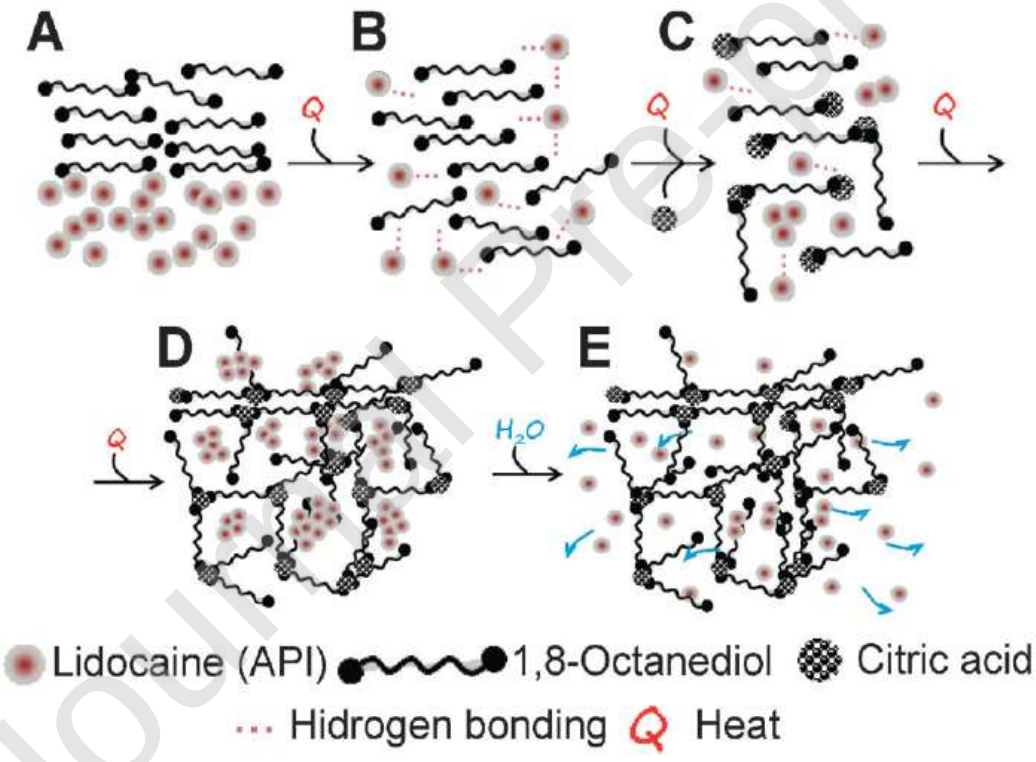


Figure 18: The synthesis process of POS, A: heterogeneous mixture of 1,8-octanediol-lidocaine in solid phase B: DES of 1,8-octanediol-lidocaine, C: lidocaine-loaded pre-polymer, D: POS, E: the release of lidocaine based on polymers biodegradation process. Reprinted with permission from Ref [131] Copyright 2012 Royal Society of Chemistry.

2.4. DES as a novel functionalizing agent for drug carriers

Recently, Hayyan group have introduced DESs as novel functionalizing agents for carbon nanomaterials (CNMs) [128], conferring surface modifications and new functional groups. The DES-functionalized CNMs demonstrated significant enhancement of their dispersion stability in various organic solvents and also in aqueous solutions [128, 129]. In the most recent work, DES has been presented as a novel functionalizing agent for application in drug delivery [130]. In this study, DES functionalization was introduced to improve the biocompatibility of graphene by providing surface modifications and hydrophilic functional groups. These features provide better dispersion and stability in the biological cellular environment, thereby enhancing the interaction between DES-functionalized graphene and biological organelles. Furthermore, DES-functionalized graphene, especially for the case of DES ChCl: malonic acid (1:1), considerably improved the cytotoxicity profile of graphene against mammalian cells, increasing the IC_{50} (>200 µg/ml) above those of pristine graphene and oxidized graphene; it also significantly increased the tamoxifen (anti-cancer drug) entrapment efficiency and loading capacity of the graphene. The hydrophilicity enhancement of DES-functionalized graphene also improved interactions between the graphene and the drug (established by hydrogen bonding, electrostatic, and Coulombian weak forces) [131, 132]. The main keys for establishing such interactions are functional groups such as oxygen groups (e.g. -OH, -COOH, and -CO) and amine groups (e.g., NH_2) introduced via DES functionalization. Subsequent drug release is more efficient because the hydrogen bonding/ Coulombian/ electrostatic forces are easily broken [133-135]. This study is a “stepping stone” that illustrates the promising avenues for use of DESs as functionalizing agents in drug delivery systems.

3. Potential biomedical and pharmaceutical applications of DESs

Recently, DESs have been implicated as potential candidates for active anti-bacterial, anti-fungal, anti-viral, and anti-cancer agents. Although the study of this field is still in preliminary stages, some positive results should be highlighted and plans made for future research. Herein, recent studies are highlighted with an emphasis on current progress (Figure 19).

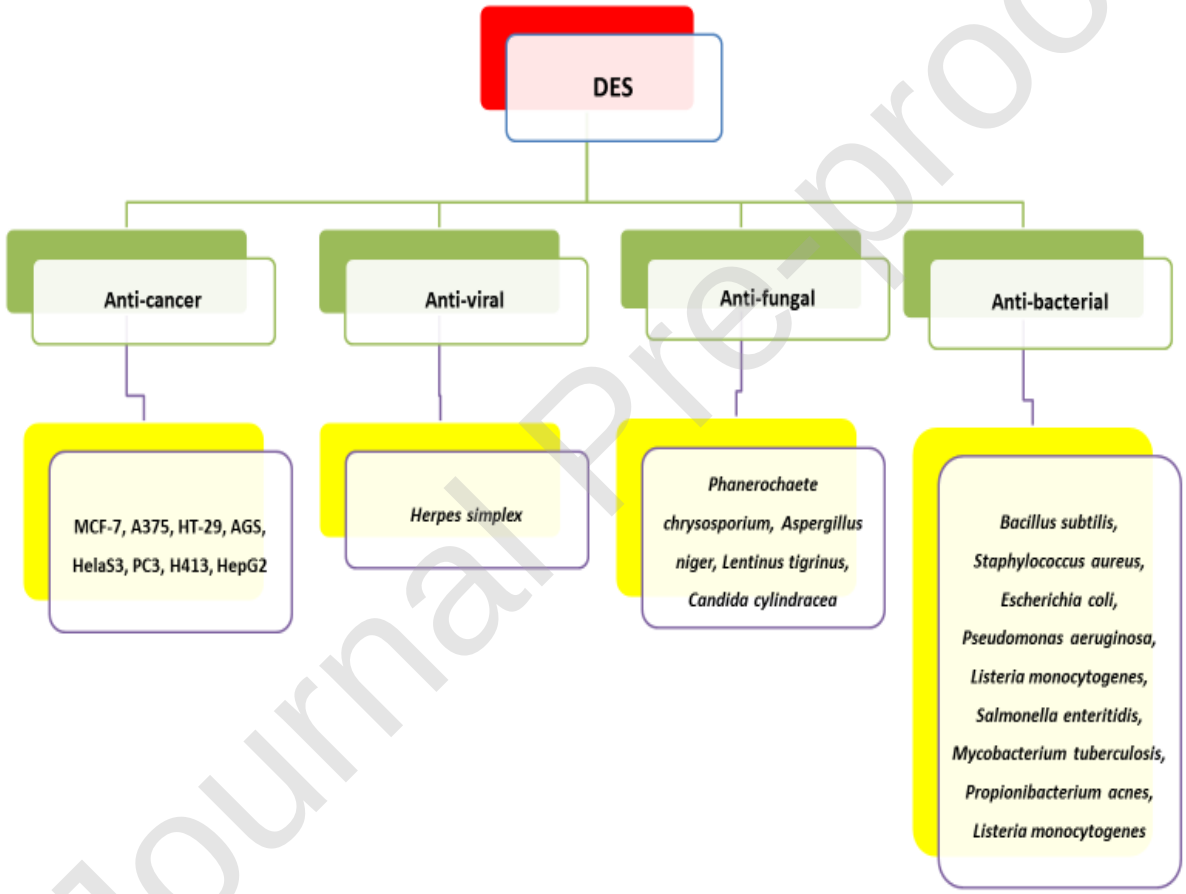


Figure 19: Promising pharmaceutical activities of DESs

3.1. Anti-microbial activities of DESs

Initial reports of DES anti-bacterial activity were made by Hayyan et al. [136, 137] and involved two sets of DESs, namely cholinium- and phosphonium-based, that were evaluated for activity against diverse bacteria species. Of the two, phosphonium-based DESs showed higher inhibition of both Gram-positive and Gram-negative bacteria (Gram-positive: *Staphylococcus aureus, Bacillus subtilis;* Gram-negative: *Pseudomonas aeruginosa, Escherichia coli*). Notably, both sets of DESs also showed higher anti-bacterial activities than their corresponding individual components. Since similar types of HBDs (i.e. triethylene glycol, glycerine, and ethylene glycol) were used in these two experiments, the results proved that the type of salt plays an important role in determining the anti-bacterial activity of a DES. Other researchers have added that DESs at lower concentrations are non-toxic to bacteria, but at higher concentrations, their anti-bacterial activities are significantly more acute than those of their individual components [138].

In another work, Zakrewsky et al. [58] reported wide-spectrum anti-bacterial activities for DES choline bicarbonate and geranic acid (CAGE, ratio 1:2) against thirty-seven different strains (Figure 20), including a pathogen resistant to diverse drugs such as ethionamide, rifampicin, mupirocin, isoniazid, carbapenem, macrolide, ciprofloxacin, doxycycline, vancomycin, erythromycin, methicillin, and cephalexin. The most promising result was its activity against *Mycobacterium tuberculosis* (TB), which is the hardiest and most multidrug-resistant of lung-infecting pathogens, and also capable of evading the human immune system [139]. Astonishingly, TB was the microbe most susceptible microbe to treatment with CAGE; less than 0.195% v/v CAGE was needed for complete pathogen neutralization. *In vivo*, CAGE also cured *Propionibacterium acnes* infection under deep skin layers. In addition, it

exhibited negligible toxicity and no signs of skin irritation when applied to human keratinocytes and mice *in vivo* [58].

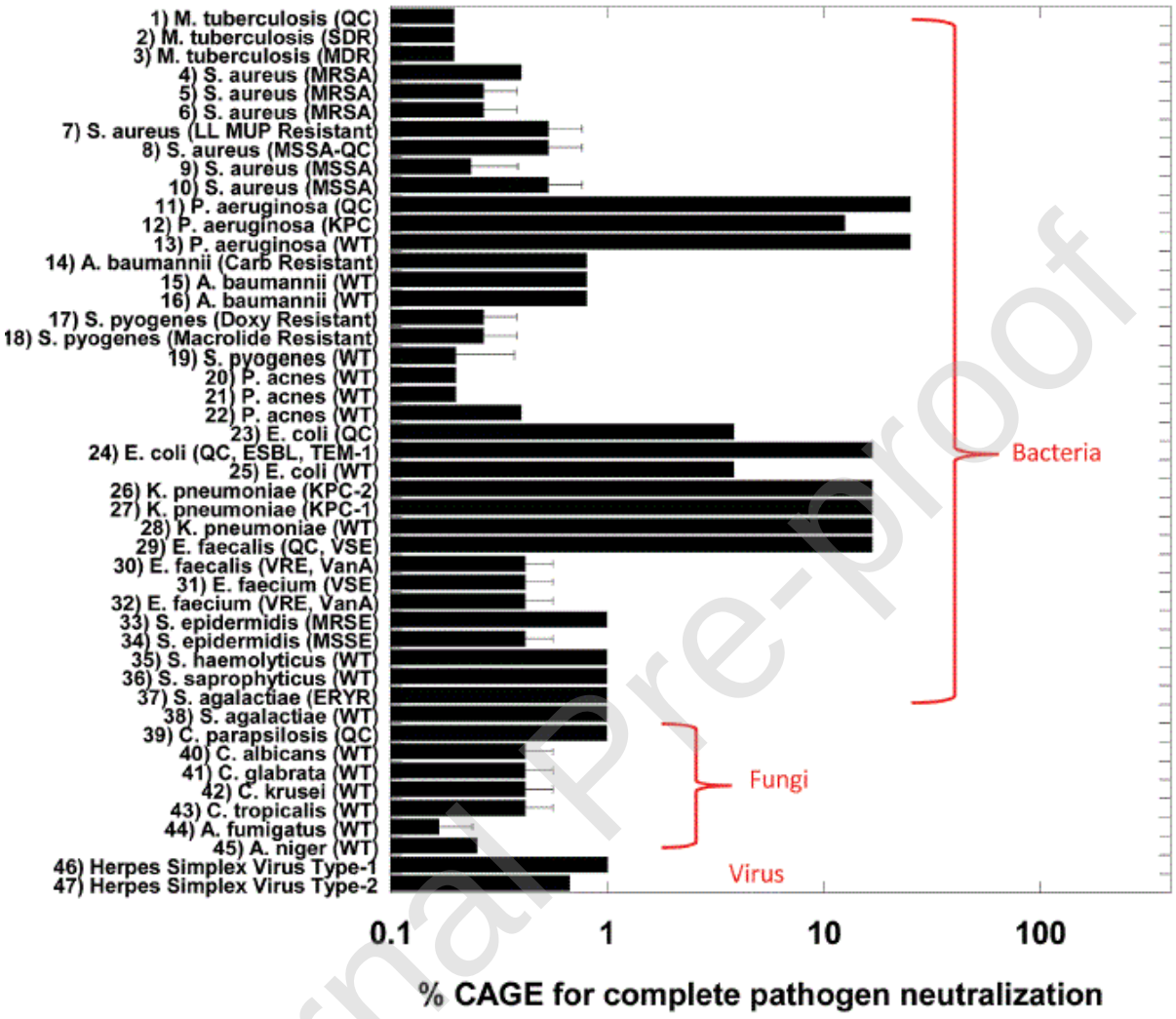


Figure 20: The wide-spectrum antimicrobial activity of CAGE towards 47 different strains of bacteria, fungi and viruses. Reprinted with permission from Ref [57] Copyright 2016 John Wiley and Sons.

In another evaluation of DES anti-bacterial activities, a number of DESs were tested against *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enteritidis* [140]. Inhibition of bacterial growth was observed for organic acid-based DESs including

ChCl:*p*-toluenesulfonic acid, ChCl:oxalic acid (1:1), ChCl:levulinic acid (1:2), ChCl:malonic acid (1:1), ChCl:malic acid (1:1), ChCl:citric acid (1:1) and ChCl:tartaric acid (2:1) (Table 7). In contrast, alcohol-based, sugar-based, and amine-based DESs exhibited no inhibition of the same bacterial species. Similar results were reported by Hayyan et al. [136] for ammonium-based DESs on the same species of bacteria towards. When using NADES, again, the organic acid-based DESs demonstrated a considerable anti-bacterial effect, while alcohol-based, sugar-based, and amine-based mixtures showed no inhibition of bacterial growth (Table 7).

Radoševi et al. [141] demonstrated that betaine-, choline-, sugar-, and sugar alcohol-based NADESs had no significant inhibition effect on tested bacteria, including *Candida albicans, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus,* and *Escherichia coli*. In contrast, citric acid-based DESs exhibited significant inhibition, with maximum inhibition observed for citric acid:glucose:glycerol (22 to 83 mm) and citric acid:fructose:glycerol (28 to 81 mm) (Table 7). This indicates that citric acid-based DESs can potentially be used as anti-bacterial agents. The anti-bacterial effect of organic acid-based DESs is generally ascribed to their acidity (pH < 6.0), which is below the optimal pH for bacterial growth (pH 6.5-7.5). The presence of hydroxyl groups in organic acid-based DESs may amplify this anti-bacterial activity [142]. Acidic HBDs may also denature the bacterial cell membrane, leading to cell death [143]. These multiple modes of action indicate that the anti-bacterial properties of DESs are highly tunable and can be regulated through controlling their ingredients.

Evaluation of ChCl-based DESs for activity against various fungi such as *Lentinus tigrinus, Phanerochaete chrysosporium, Candida cylindracea*, and *Aspergillus niger* revealed the

highest anti-fungal activity for ChCl:zinc chloride (1:2) exhibited, with inhibition zones >16 mm for all four fungi species, followed by ChCl:malonic acid and (1:1) and DES ChCl:*p*-toluenesulfonic acid (1:3), which had average inhibition zones of 6.75 mm and 4.75 mm, respectively [144]. Additionally, the DESs had higher anti-fungal activities than did their respective starting materials. This finding is consistent with previous work [9], where ChCl:ethylene glycol (1:2) showed higher anti-fungal activity against *Aspergillus niger* than its individual starting materials. The minimum inhibitory concentration (MIC) for ChCl:ethylene glycol was 325.3 mg/L, whereas those for ethylene glycol and ChCl alone were 322.7 mg/mL and 628.2 mg/mL, respectively. The differences in anti-fungal activity between DESs and their individual components are probably due to the synergistic effects resulting from DES formation [137]. Another possible factor is hydrogen bonding between the salt anion and HBD, which may change the physicochemical properties of the mixture [145]. Specifically, the charge delocalization that develops through hydrogen bonding is expected to cause the mixture to be more toxic [146].

A comparison of ChCl-based and *N*,*N*-diethylethanolammonium chloride (EAC)-based DESs revealed higher anti-fungal activity for the latter against *Aspergillus niger* [9]. The highest anti-fungal activities were observed for EAC:zinc chloride (1:2) and EAC:zinc nitrate hexahydrate (1:1), with MIC values of <1.3 mg/mL and <2.2 mg/mL, respectively. EAC:malonic acid (1:1) followed with a MIC of 64.42 mg/mL. Juneidi et al. [9] ascribed that DES types I, II, and IV, which contain metal salts that interact ionicially, have higher anti-fungal activities than do type III DESs, which contain amides and polyols such as urea, glycerol, and ethylene glycol. Details on the classification of all types of DES can be obtained from [8]. Meanwhile, a mild anti-fungal effect was observed for ChCl:urea (1:2), with MIC 138.5 mg/mL [9]. This is because the urea is not toxic to the fungi and might even

be used as a fertilizer and nitrogen source for fungal growth [147, 148]. The mechanism of DES toxicity towards fungi is speculated to involve stimulating dehydrating effects, similar to those induced by $CaCl_2$ (a dehydrating agent) [149, 150]. However, further work is greatly needed to unravel the mechanism of DES anti-fungal activity in order to strategize the application of DESs as anti-fungal agents.

A recent study [58] has shown that DES CAGE possesses anti-viral activities against two types of viruses, namely herpes simplex virus type-1 (HSV-1) and herpes simplex virus type-2 (HSV-2), via a neutralization mechanism. Specifically, HSV-1 and HSV-2 were completely neutralized from infecting the cells at <1.0% CAGE, as confirmed by the absence of viral plaques after incubation (Figure 21). However, this finding was not discussed further, which leaves unanswered many questions pertaining to the anti-viral activity of CAGE. For instance, virus neutralization mechanisms normally involve the specific binding of antibody to the virus receptor [151-154]; how does the neutralization of HSV-1 and HSV-2 by CAGE occur? Is there any specific binding to the HSV-1 and HSV-2 receptors? Therefore, there is still need for additional assessment and verification of the mechanism of action for CAGE, along with elucidating the interactions between CAGE and the viruses. Additionally, investigations into the anti-viral effects of DESs should be extended to other families of pathogens. It is important that the anti-viral DESs be well-characterized, including their biological activities on other, non-pathogenic microorganisms. Although only one study has reported on the anti-viral activity of a DES, its promising results may prompt the scientific community to continue the effort and pursue all stages of anti-viral validation, including *in vitro*, *in vivo*, and pre-clinical.

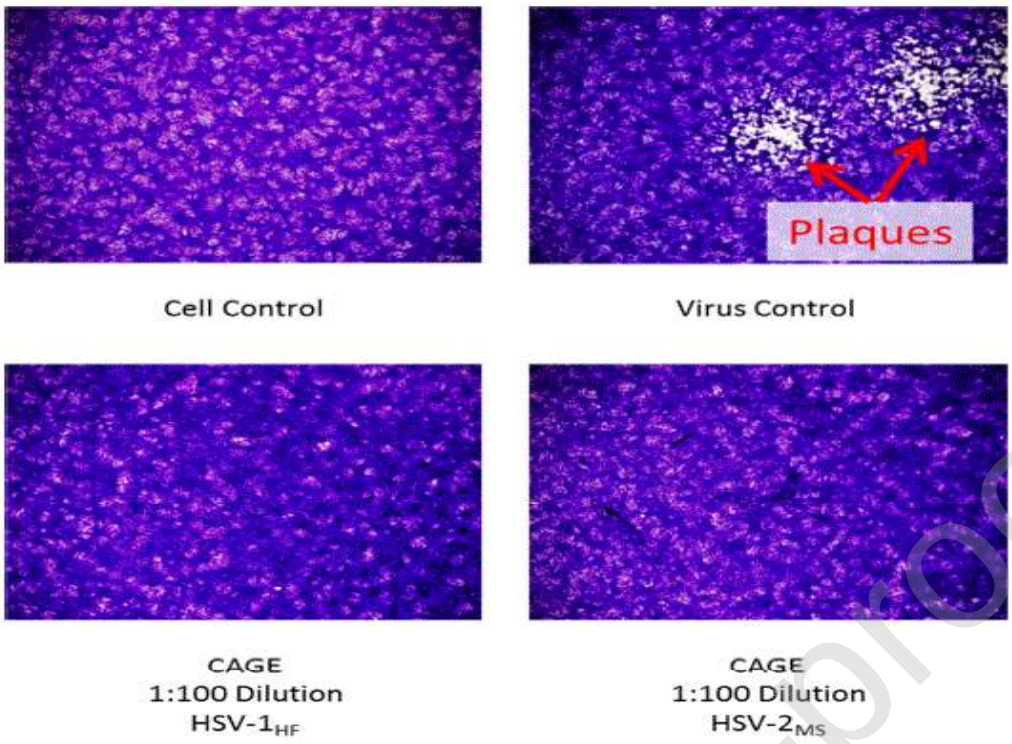


Figure 21: HSV-1 and HSV-2 were neutralized by CAGE. Reprinted with permission from Ref [61] Copyright 2016 John Wiley and Sons.

Overall, the anti-microbial activities (i.e. anti-fungal, anti-bacterial, and anti-viral) of DESs show great promise for the medical and pharmaceutical fields. While the high toxicity of DESs against microorganisms may impede their applications in biotechnology, they may be useful in pharmaceutical applications. Figure 22 depicts the advantages and disadvantages of using DESs as anti-microbial agents. Notably, the toxicity of DESs against non-pathogenic species is a serious concern, and therefore the investigation and optimization of appropriate DESs for medical and pharmaceutical applications are highly recommended. As with other available anti-microbial agents, the broad application of DESs may possibly instigate the appearance of resistant strains. Although no evidence has yet been shown for DESs, their analogues, ILs, have been reported to stimulate the expression of antibiotic resistance genes

and also to trigger horizontal gene transfer in freshwater bacteria, which eventually facilitates the transmission of antibiotic resistance genes information between different species of microbes [155]. This potential negative effect should be paid full attention by the scientific community.

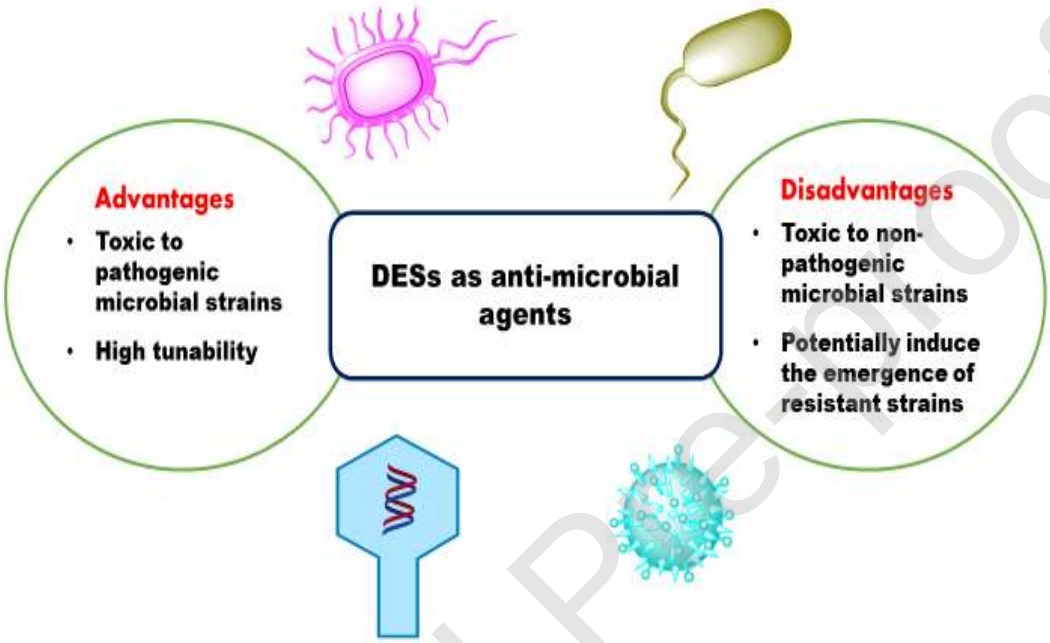


Figure 22: Graphical summary of DESs as anti-microbial agents.

Table 7: Anti-microbial activities of DESs/NADESs against various strains.

DESs/NADEs	Inhibition (in cm)										Reference
	L. monocytogenes	*S. aureus*	*S. enteritidis*	*E. coli*	*P. aeruginosa*	*B. subtilis*	*P. mirabilis*	*S. typhimurium*	*A. niger*	*C. cylindracea*	
Betaine:malic acid:glucose (1:1:1)	–	5.10, 4.70, 4.40	–	2.80, 2.30	5.00, 4.50, 2.80	–	7.30, 6.50, 4.40	3.00, 2.80, 3.40	–	–	[146]
Betaine:malic acid:proline (1:1:1)	–	4.90, 4.50, 4.40	–	4.40, 2.20	4.50, 3.80, 3.00	–	6.20, 4.50, 4.70	2.40, 2.70	–	–	[146]
ChCl:1,4-butanediol (1:4)	NI	NI	NI	NI	–	–	–	–	–	–	[145]
ChCl:acetamide (1:2)	NI	NI	NI	NI	–	–	–	–	–	–	[145]
ChCl:citric acid (1:1)	1.30	1.58	1.77	1.93	–	–	–	–	–	–	[145]

ChCl:citric acid (1:1)	1.30	1.58	1.77	1.93	–	–	–	–	_	_	[145]
ChCl:D-sorbitol (1:1)	NI	NI	NI	NI	–	–	NI	NI	_	_	[145]
ChCl:ethylene glycol (1:2)	NI	NI	NI	NI	–	–	–	–	_	_	[141]
ChCl:fructose:water (5:2:5)	NI	NI	NI	NI	–	–	–	–	_	_	[145]
ChCl:glucose:water (5:2:5)	NI	NI	NI	NI	–	–	–	–	_	_	[145]
ChCl:glycerol (1:2)	NI	NI	NI	NI	–	–	–	–	_	_	[145]
ChCl:levulinic acid (1:1)	0.97	1.00	1.60	1.65	_	_	–	–	_	_	[145]
ChCl:levulinic acid (1:2)	0.97	1.00	1.60	1.65	–	–	–	–	_	_	[145]

ChCl:malic acid (1:1)	1.10	1.50	1.22	1.92	–	–	–	–	_	_	[145]
ChCl:malonic acid (1:1)	0.93	1.32	1.17	1.53	–	–	–	–	~0.68	~0.68	[145, 149]
ChCl:maltose:water (5:2:5)	NI	NI	NI	NI	–	–	–	–	_	_	[145]
ChCl:oxalic acid (1:1)	1.50	1.97, 5.00, 5.50, 5.00	1.93	2.48	5.00, 4.90, 4.90	–	4.90	4.50	_	_	[146]
ChCl:oxalic acid (1:1)	1.50	1.97	1.93	2.48	–	–	–	–	_	_	[145]
ChCl:*p*-toluenesulfonic acid (1:1)	0.70	1.12	1.20	1.71	–	–	–	–	–	_	[145]
ChCl:*p*-toluenesulfonic acid	–	–	–	–	–	–	–	–	~0.48	~0.48	[149]

(1:3)										
ChCl:sucrose:water (5:2:5)	NI	NI	NI	NI	–	–	–	–	_	_	[145]
ChCl:tartaric acid (1:1)	1.10	1.50	1.50	1.76	–	–	–	–	_	_	[145]
ChCl:tartaric acid (2:1)	1.10	1.50	1.50	1.76	–	–	–	–	_	_	[145]
ChCl:triethylene glycol (1:4)	NI	NI	NI	NI	–	–	–	–	_	_	[141]
ChCl:urea (1:2)	NI	NI	NI	3.70	–	NI	NI	NI	_	_	[141, 145]
ChCl:xylitol (1:1)	NI	NI	NI	NI	–	–	NI	NI	_	_	[145]
ChCl:xylose:water (1:1:1)	NI	NI	NI	NI	–	–	–	–	_	_	[145]

ChCl:zinc chloride (1:2)	–	–	–	–	–	–	–	–	>1.6	>1.6	[149]
Citric acid:fructose:glycerol (1:1:1)	–	5.10, 4.80, 4.30	–	5.00, 4.50, 3.80	5.10, 4.30, 2.80	–	8.10, 7.60, 5.00	5.50, 4.20, 3.70	–	–	[146]
Citric acid:glucose:glycerol (1:1:1)	–	4.60, 4.40	–	3.40, 2.20	4.70, 4.40	–	8.30, 4.90	5.10, 5.70	–	–	[146]
Citric acid:proline (1:1)	–	5.00, 4.60, 4.40, 4.80	–	4.90, 4.40, 3.30, 4.60	4.40, 4.50, 2.40, 5.00	–	4.70, 5.60, 4.40, 6.80	3.30, 3.70, 2.60, 4.40	–	–	[146]
MTPPB:ethylene glycol (1:3)	–	0.15	–	0.70	0.45	0.75	–	–	–	–	[142]

MTPPB:glycerol (1:3)	–	NI*	–	NI	0.70	NI	–	–	–	–	[142]
MTPPB:triethylene glycol (1:3)	–	0.30	–	0.50	0.35	NI	–	–	–	–	[142]

Abbreviation: ChCl (choline chloride), no inhibition (NI), methyltriphenylphosphonium bromide (MTPPB

3.2. Anti-cancer activity

Although research is still in preliminary stages, the reported anti-cancer behavior of DESs is promising [18, 145, 156]. It has been agreed that the complexation of two or more components in DESs could produce more acute destructive impacts on cancer cells, compared to the individual components alone [4]. Ammonium-based DESs have been evaluated for selectivity towards cancer cell lines and a normal cell line (OKF6, human oral keratinocytes) [145]. The selectivity index values of the ammonium-based DESs varied across the range of > 2 or ≤ 2 (Table 8). ChCl:glycerine (1:3) was found to be selective against a human malignant melanoma cell line (A375) and a human breast cancer cell line (MCF-7); ChCl:ethylene glycol (1:3) was selective against a human colon adenocarcinoma cell line (HT-29), a human prostate cancer cell line (PC3), a human liver hepatocellular cell line (HepG2), and MCF-7 cells; ChCl:urea (1:3) was selective against HT29, PC3, HepG2 and MCF-7 cells; and ChCl:triethylene glycol (1:3) was selective against A375 and MCF-7 cells (Table 8). All DESs displayed low selectivity indexes for the carcinoma-derived human oral keratinocyte cells (H413). Other synthetic anti-cancer drugs, such as alisiaquinol, 4-hydroxy tamoxifen and piperidinyl-diethylstilbestrol, have shown lower selectivity indexes (i.e. < 2) [157]. However, further investigation is recommended in order to find other DESs with higher selectivity. Based on the aforementioned results, the existence of DESs with acute toxicity against cancerous cells while being harmless to non-cancerous cells is not implausible. Better understanding of the mechanism of DES toxicity against cancer cells may generate new ideas for developing a DES tailor-made for anti-cancer applications.

Table 8: Selectivity index of ammonium–based DESs for cancer cells compared to human oral keratinocyte cell line. Reprinted with permission from Ref [150] Copyright 2015 PLOS.

DES	Selectivity Index					
	MCF-7	PC3	A374	HepG2	HT29	H413
ChCl:glycerine	2.162	1.542	2.615	1.309	1.662	0.864
ChCl:ethylene glycol	2.579	2.120	1.979	2.812	2.282	1.232
ChCl:urea	2.789	2.949	1.374	2.172	2.263	1.204
ChCl:triethylene glycol	2.137	1.692	2.797	1.902	1.974	1.782

It has been implied that one mechanism by which DESs induce cancer cell death is through disruption of the cell membrane. Cell membrane disruption was observed in three different cancer cell lines (MCF-7, a human gastric cancer cell line [AGS], and a human cervical cancer cell line [HelaS3]) when the permeability dye stained-cells were treated with ChCl:fructose (2:1), ChCl:glucose (2:1), and DAC:TEG (1:3) (Figure 23). In another study on ammonium-based DESs, the cell membranes of MCF-7 cells were disrupted, as evidenced by increased release of lactate dehydrogenase (LDH). The increase occurred in a dose-dependent manner for DESs in the concentration range of 12.5 µg/mL–50 µg/mL. MCF-7 cells treated with ChCl:triethylene glycol (1:3) exhibited the highest LDH-release (~45–70%). However, there was no DNA degradation observed after treatment with DESs. This

indicates that the cell death mechanism for ammonium-based DESs is not through DNA fragmentation.

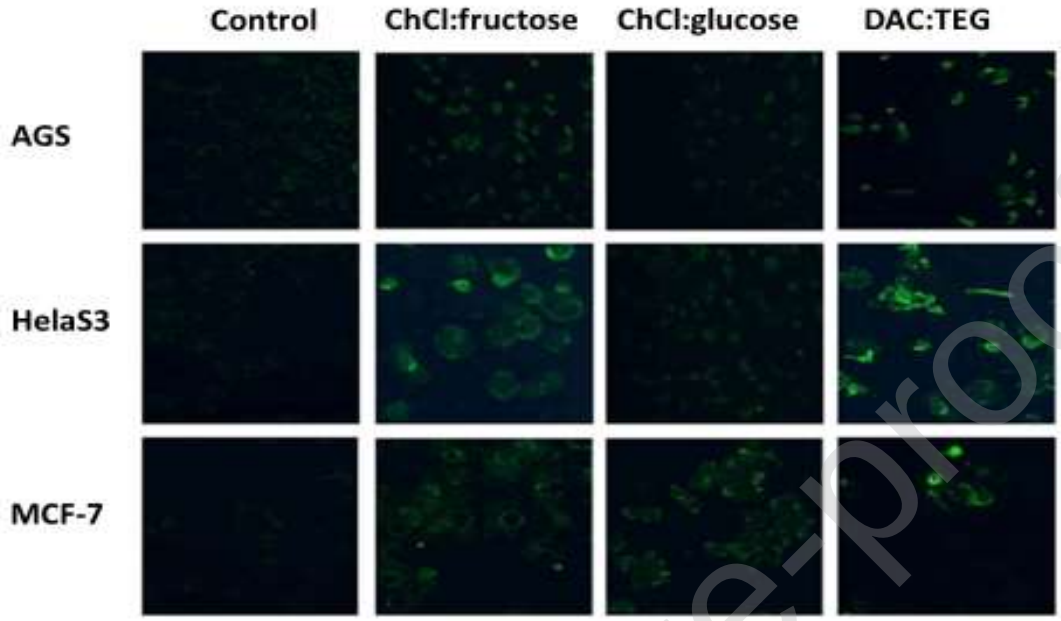


Figure 23: Effect of DESs (i.e., ChCl:fructose, 2:1; ChCl:glucose, 2:1 and DAC:TEG, 1:3) on the cell membrane permeability of various cancer cell lines (i.e., MCF-7, AGS and HelaS3). Reprinted with permission from Ref [161] Copyright 2017 Nature.

As a result of the cell membrane disruption in MCF-7 cells, reactive oxygen species (ROS) generation is increased, stimulating apoptosis (Figure 24) [145]. Increased ROS generation was also observed in other cancer cells, such as WRL-68, HelaS3, and AGS, when they were treated with ChCl:glucose (2:1), ChCl:fructose (2:1), and diethylethanolammonium chloride:triethylene glycol (1:3) (DAC:TEG) [156]. When comparing the anti-cancer effects of NADESs and DESs, cells treated with DES DAC:TEG (1:3) exhibited more acute redox

stress than those treated with NADES ChCl:fructose (2:1) and ChCl:glucose (2:1). Based on the MTT cell viability analysis, DES DAC:TEG (1:3) also exhibited higher anti-cancer activity compared to NADES ChCl:fructose (2:1) and ChCl:glucose (2:1) [18, 156]. When tested on a variety of cancer cells such as PC3, HelaS3, AGS, A375, and MCF-7 cells, the IC_{50} of DESs was in the range of $34 \leq IC_{50} \leq 120$ mM, which is more toxic than those of NADESs (range $98 \leq IC_{50} \leq 516$ mM).

DESs also may initiate cancer cell destruction through changing the cell morphology. As depicted in Figure 25, cancer cells treated with ChCl:fructose (2:1), ChCl:glucose (2:1), and DAC:TEG (1:3) display lethal impacts to their morphology [156], with the cells being shrunk significantly and the field filled with many dead cells. Again, DES DAC:TEG (1:3) demonstrated more pronounced destructive effects than did NADES ChCl:glucose (2:1) and ChCl:fructose (2:1).

The weaker anti-cancer effects of NADESs are expected due to their natural-origin starting materials, for which cellular tolerances are high. For example, glucose and fructose are utilized as sources of energy and carbon for cell proliferation. Glucose is basically metabolized through glycolysis to produce energy, and also provides metabolic intermediates for other cellular pathways such as the pentose phosphate pathway and tricarboxylic acid pathway [158]. Cancer cells require more nutrients (e.g. sugars, salts, and amino acids) due to higher energy demands for their cell growth. This is probably the key reason for the greater cellular tolerance of ChCl:fructose (2:1) and ChCl:glucose (2:1) by cancer cells in comparison to DAC-TEG (1:3).

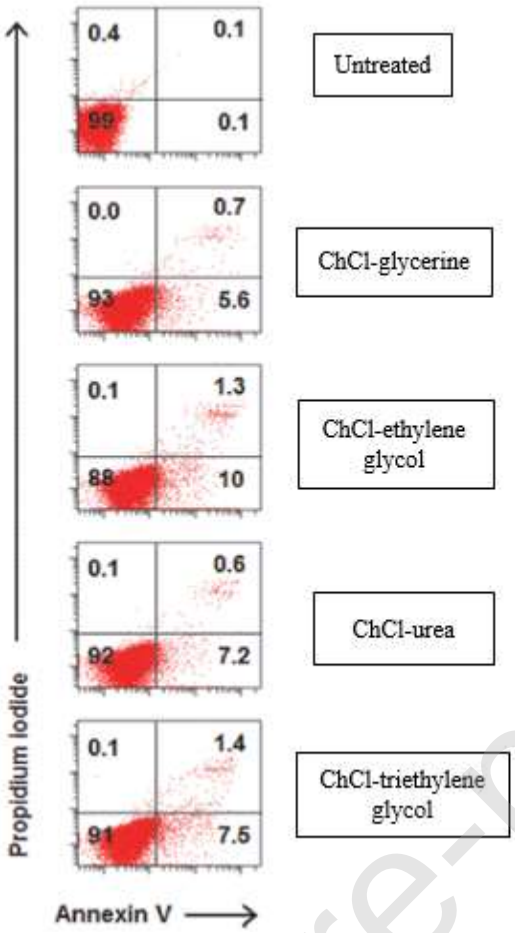


Figure 24: Apoptotic assay was determined in MCF-7 cells treated with ammonium-based DESs at concentration 100 µg/mL for 48 h. Cells were marked with propidium iodide (PI) and annexin V in order to evaluate the percentages of live cell (PI+ annexin V+), early apoptotic cells (PI– annexin+), and late apoptotic cells (PI+ annexin V+). Reprinted with permission from Ref [150] Copyright 2015 PLOS.

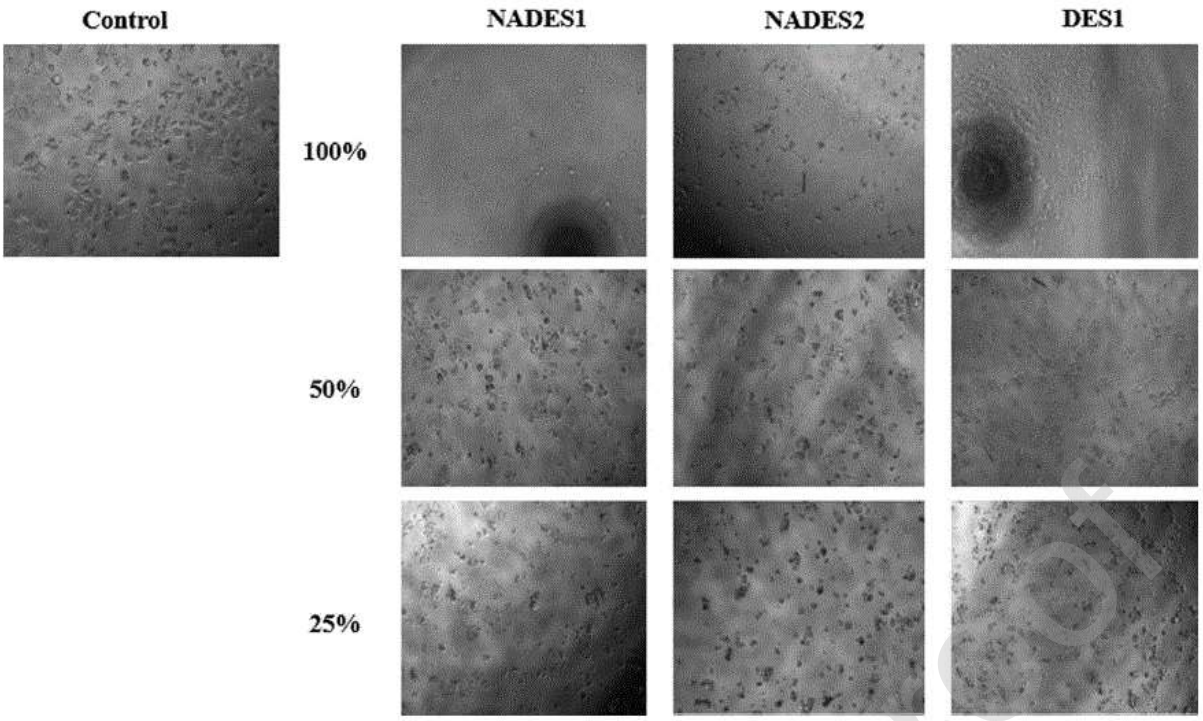


Figure 25: Light microscope images of MCF-7 cells treated with DES/NADESs. Control cells indicate the cells without any treatment and being considered as the 100% growth. The other cells were treated with ChCl:fructose (2:1) (NADES 1) and ChCl:glucose (2:1) and diethylethanolammonium chloride:triethylene glycol (1:3) (DES 1) at various concentration (i.e., 25%,50%, 100%) with 100% represent 4.5M [161]. Reprinted with permission from Ref [161] Copyright 2017 Nature.

Recently, for the first time, a computational simulation approach using the conductor-like screening model for real solvent (COSMO-RS) analysis has been applied to understand the interaction between NADESs and cancer cell membranes [18]. COSMO-RS is a program for the quick prediction of thermophysical and chemical attributes of solvents through analysis of the screening charge density (sigma, σ)-profile and σ-potential [159]. From this analysis, the hypothetical thermodynamic behavior of an individual compound in a solvent and its affinity towards other materials/compounds could be derived. The analysis of NADESs proposed that

mixtures such as ChCl:glucose:water (5:2:5), ChCl:fructose:water (5:2:5), and ChCl:sucrose:water (4:1:4) have strong interactions with cell membrane surfaces, and their aggregation or accumulation on the cell membrane may possibly destroy the cancer cells. However, another two NADESs, ChCl:malonic acid (1:1) and ChCl:glycerol:water (1:2:1), showed only mild interaction with the cell membrane. Their anti-cancer effects are perhaps not mainly attributable to aggregation at the cell surface, but instead through other mechanisms such as ROS generation or DNA degradation.

The anti-cancer activities of DESs are highly correlated with their starting materials, the combination of hydrogen bond acceptor and HBD, and the cell type used. For example, organic acid-based NADESs exhibited higher anti-cancer activities against two cancer cell lines (HeLa and MCF-7 cells) than did non-organic acid-based NADESs [141]. This finding is in accordance with the results obtained by Hayyan et al. [18], in which higher anti-cancer activities were observed for organic acid-based NADES (i.e. malonic acid) than sugar-based NADESs. Nevertheless, the anti-cancer activity of NADES betaine:malic acid:proline (1:1:1) was lower than other NADESs. This is probably due to suppression of the toxic potential of malic acid by betaine and proline, highlighting the tunability of DESs. The adjustable/tunable properties of DESs are their main feature in the context of creating therapeutic anti-cancer agents with selective destructive effects towards targeted cancerous cells. However, the toxicity mechanisms of DESs against both malignant and non-malignant cells are still poorly understood. Therefore, further studies are required in order to make it possible to design novel DESs with selective and targeted anti-cancer properties.

4. Biosafety aspects of DESs

The most commonly applied method in the preparation of DESs is a combination of mixing and heating [4, 160]. In this method, DES formation results from the mixing of the salt/HBA and HBD compounds while heating at temperatures below 100 °C [7, 8]. DESs also can be prepared using the grinding method, in which the DES's initial ingredients are ground using a mortar and a pestle at room temperature until a clear liquid is formed [161]. Freeze-drying is a method infrequently used for the preparation of DESs. In this method, aqueous solutions of the DES's initial ingredients are mixed and frozen, then freeze-dried to form a homogenous viscous liquid [162]. Overall, DES preparation may only take 30 min to 6 h depending on the initial ingredients and composition of the DES [19, 163, 164].

It has been attested that DESs are noticeably more easily synthesized than are ILs [165]; the preparation of DESs is also considered "greener" and low-cost. The starting materials for preparing DESs are inexpensive and also widely available. The preparation involves simple procedures, requires no purification process, and also requires no organic/volatile solvents. A comparison of the preparation processes for ILs and DESs is illustrated in Figure 26.

Before implementing DESs in drug delivery applications, a complementary investigation of toxicology profiles is indispensable to ensure their biosafety and health impacts. The following paragraphs highlight prior reports that assessed the toxicological behaviors of DESs on different organisms and environments.

The first study of DES cytotoxicity [136] revealed lethal effects for cholinium-based DESs against an aquatic organism, *Artemia salina* (brine shrimp). The DESs also showed higher

toxicity against *Artemia salina* than did their individual ingredients. Similar findings were also observed for phosphonium-based DESs against the same aquatic organism [137]. In contrast, other reports [9, 138, 144] indicated that DESs were less toxic than their individual ingredients on the European carp, *Cyprinus carpio*, and also the fresh-water organism *Hydra sinensis*. For example, the lethal concentration at 50% (LC_{50}) of DES ChCl:glycerine on *C. carpio* fish (>8,000 mg/L) was considerably higher than those of its aqueous individual ingredients [9]. This indicates that the synergistic interaction between the salt and HBD during DES formation not only changes the physical properties of the individual ingredients but also their chemical properties towards different organisms.

Although cholinium-based DESs (i.e. ChCl:glycerine and ChCl:ethylene glycol) had lethal effects on *Artemia salina*, the survival time of (> 3.25 min) was longer than for methyltriphenylphosphonium bromide (MTPPB)-based DESs (i.e. MTPPB:glycerine and MTPPB:ethylene glycol) [136]. However, ChCl-based DESs possessed greater toxicity against *H. sinensis* than those based on choline acetate (ChAc) salt [138]. This implies that the type of HBA/salt has a significant influence on the ecotoxicity profile of a DES. Such synergistic influence was also observed in a mixture toxicity assessment model using *Aliivibrio fischeri* [166, 167]. As previously mentioned [168-170], the type of HBD, the type of salt, and the molar ratio have great effects on the DES viscosity. The high viscosity of MTPPB-based DESs may be responsible for their high toxicity against brine shrimps, causing difficulty of movement and a lack of oxygen. By harmonizing the features of DES constituents, its toxicity may possibly be reduced.

In an investigation of DES toxicity towards plants (common wheat, *Triticum aestivum*), DES ChCl:glycerine and ChCl:glucose exhibited low phytotoxicity in all evaluated parameters, with an effective concentration 50% (EC_{50}) >20,000 mg/L; this is in contrast to the low EC_{50} of DES ChCl:oxalic acid (i.e. EC_{50} > 5,325.26 mg/L) [171]. The toxicity of ChCl:oxalic acid can be attributed to its acidity (pH: 2.08). Interestingly, the results from all tested DESs showed no significant inhibition of seed germination.

Work done by Wen et al. [138] provided evidence that ChCl- and ChAc-based DESs induce morphological distortion of garlic plants (*Allium sativum*) (Figure 27). However, treatment with DESs induced a significant improvement in root growth as compared to their individual ingredients, especially for the case of DES ChCl:glycerol (1:1). This indicates that the hydrogen bonding interaction between ChCl and glycerol in DES form can ameliorate their toxicity towards *Allium sativum.*

An *in vitro* study on human embryonic kidney cells (HEK-293) revealed that DES formation resulted in increased toxicity level compared to the individual (IC_{50} values: 3.52-75.46 mM) [172]. Nevertheless, DES toxicity was still significantly lower than that of the most extensively used IL, 1-octyl-3-methy-limidazolium chloride ([C_8mim][Cl]), which has an IC_{50} of 0.02 mM. This is in a good agreement with a previous report on the potential toxicity of ILs and DESs [173]. Meanwhile, the addition of water into organic acid-based DESs has been shown to reduce their cytotoxicity on HEK-293 cells [174]. This implies that the addition of an appropriate amount of water may reduce DES viscosity, hence ameliorating its cytotoxicity profile.

As a closer representation of toxicity in therapeutic use, the *in vivo* toxicity of DESs on mice was investigated by Hayyan et al. [145]. Their results showed that ChCl-based DESs had more lethal effects on mice than did the corresponding individual ingredients. For example, DES ChCl:ethylene glycol (1:3) had a median lethal dose (LD_{50}) value of 5.33 g/kg, whereas the LD_{50} values for pure ethylene glycol and pure ChCl were 9.71 g/kg and >20 g/kg, respectively. This pattern of relative toxicity is similar the results observed with brine shrimp (*Artemia salina)* and a normal human cell line (HEK-293), but contradicts the results from marine organisms (*C. carpio* and *H. sinensis*) and garlic (*Allium sativum*). This study also highlighted that ChCl-based DESs considerably increased the level of aspartate transaminase (AST) (by 5.7- to 9.5-fold) while maintaining normal levels of alanine aminotransferase (ALT) and alkaline phosphatase in liver serum. This alteration in the ratio of AST:ALT suggests that a hepatocellular form of liver injury occurred following DES treatment.

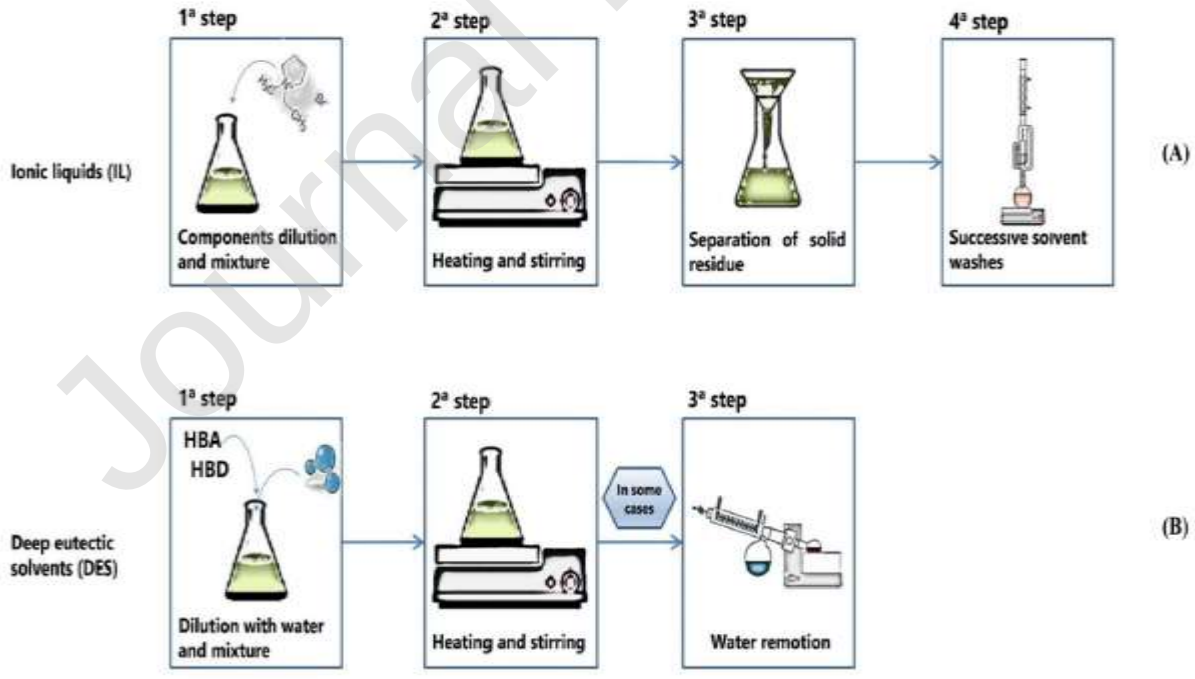


Figure 26: The preparation process of ILs (A) and DESs (B). Reprinted with permission from Ref [170] Copyright 2019 Elsevier.

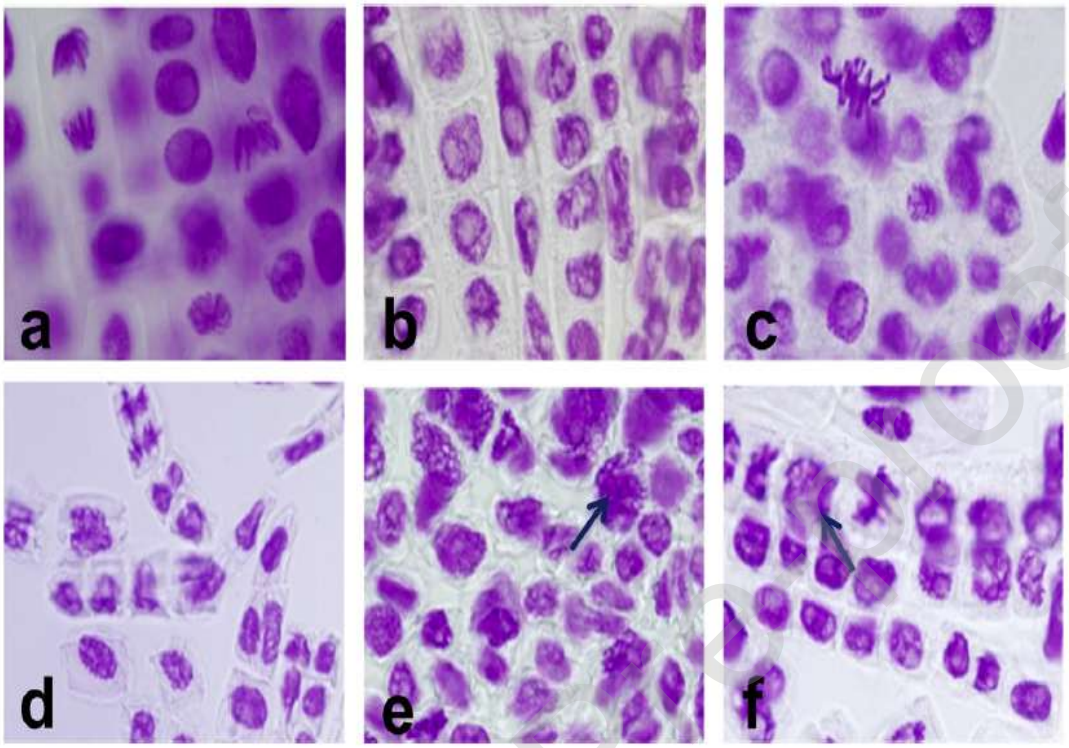


Figure 27: Optical microscopy images of the root tip cells of *A. sativum* treated with (A) deionized water, (B) ChCl, (C) ethylene glycol, (D) ChAc:glycerol,(1:1) (E) ChAc:urea,(1:1) and (F) ChCl:acetamide (1:1). Reprinted with permission from Ref [143] Copyright 2015 Elsevier.

5. Conclusion and future directions

Eutectic systems, including DESs, are deemed to have huge potential for beneficial applications in drug delivery owing to their potential to increase drug dissolution, improve drug penetration, and act as a synthesis medium for drug carriers. Examples of the application of eutecticity in drug delivery include acting as drug solubilization vehicles, polymeric

eutectic delivery systems, dual-drug eutectic systems, topical drug carriers, and producing microemulsion and drug-based DESs.

Most existing studies of eutectic-based drug carrier development systems have focused on ibuprofen as the subject. Different drugs will certainly have different behaviors or mechanisms of action. Thus, more studies on other types of drugs are required to explore the potential application of eutectic systems as a universal drug delivery system that is not limited to any one particular type of drug.

There is a lack in systematic comparative studies that dedicated to different types of drug-based DESs or THEDES concerning their applications as drug delivery systems. These studies will be beneficial in identifying the optimum DES for drug delivery and avoiding any conflicts or uncertain results that stem from different research groups. This can be approached through the integrative effort of the scientific community.

The functional mechanisms of many drugs/APIs incorporated in eutectic systems are poorly understood and merit further exploration. An underlying question concerns the identity of the incorporated drug/API after formation of the eutectic mixture. It is known that at the eutectic point, the mixture of drugs and excipients forms a new identity (i.e. the eutectic mixture or DES), which has physicochemical properties and behaviors that differ from its components' original properties. Therefore, the question remains, are the biological activities of the mixture due to the drugs or due to the collective identity of the eutectic mixture? Further investigation is highly essential to find out the answer(s).

It has been speculated that eutectic mixtures may enhance drug penetration through the skin by means of disturbing the skin's structure. However, there is a scarcity of knowledge on the effect of eutecticity towards the skin membrane, which should receive full attention from the scientific community. Further investigation, such as electron micrograph analysis of the skin

membrane, could be done before and after the application of drug-based eutectic systems. The best drug vehicle is the one that causes reversible membrane damage (temporary damage), which means the cells can regain membrane integrity after treatment.

In addition to drug delivery applications, certain types of DESs have been discovered to possess medicinal or pharmaceutical activities (e.g. anti-bacterial, anti-fungal, anti-viral, and anti-cancer activities). Although the *in vitro* preliminary results are promising, these are still far from clinical applications, with further investigation being needed prior to clinical use. Future directions are to continue developing in-depth studies on the potential pharmaceutical activities of DESs and to establish a targeted DES-based drug delivery system.

With regards to regulatory aspects, DESs are being widely explored and have thus far demonstrated mild toxicity towards biological environments. However, there remains a need to ensure that the findings are in line with strict regulation and the standards set by international and governmental drug organizations.

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